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UNION OF
SOUTH AFRICA.

DEPARTMENT OF
AGRICULTURE.

ANNUAL REPORT

of the

Director of Veterinary Services,

Onderstepoort, Pretoria.

15th Report

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DEPARTMENT OF AGRICULTURE,
DIRECTOR OF VETERINARY SERVICES,
ONDERSTEPOORT LABORATORIES,
PRETORIA, SOUTH AFRICA,
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Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1903–4.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1904–5.*
Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1905–6.*
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Report of the Government Veterinary Bacteriologist of the Transvaal for the year 1907–8.*
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First Report of the Director of Veterinary Research, August, 1911.*
Second Report of the Director of Veterinary Research, October, 1912.*
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Fifteenth Report of the Director of Veterinary Services, October, 1929.

P. J. DU TOIT,
Director of Veterinary Services.

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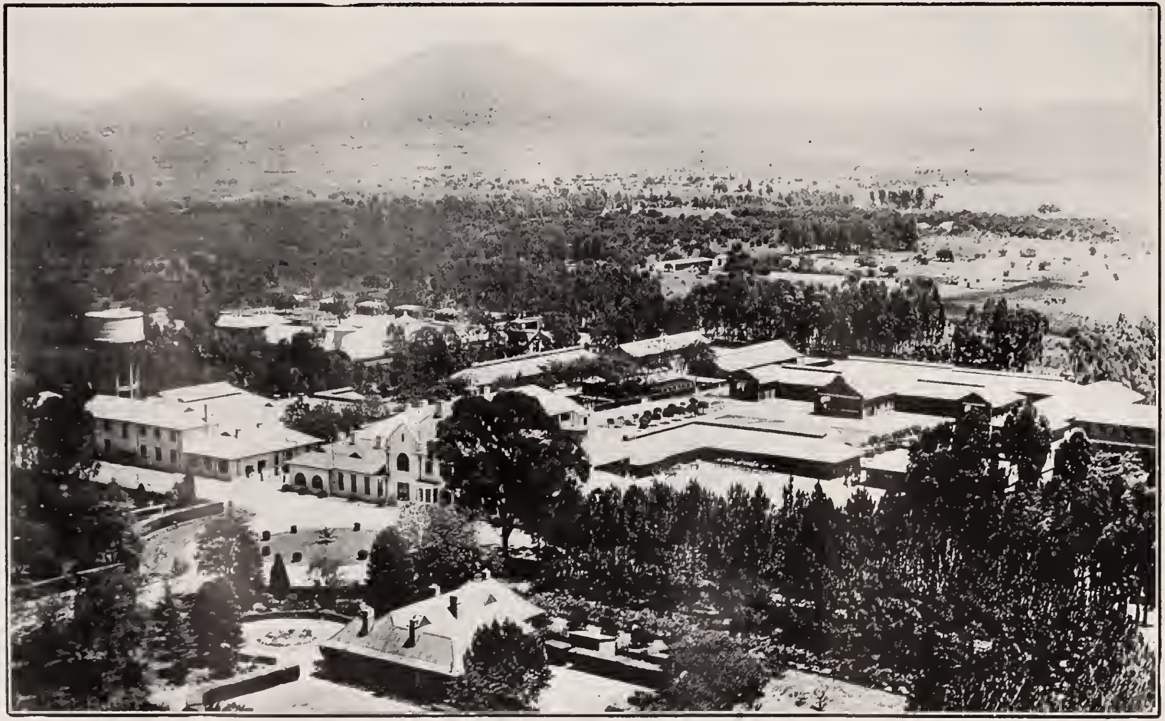
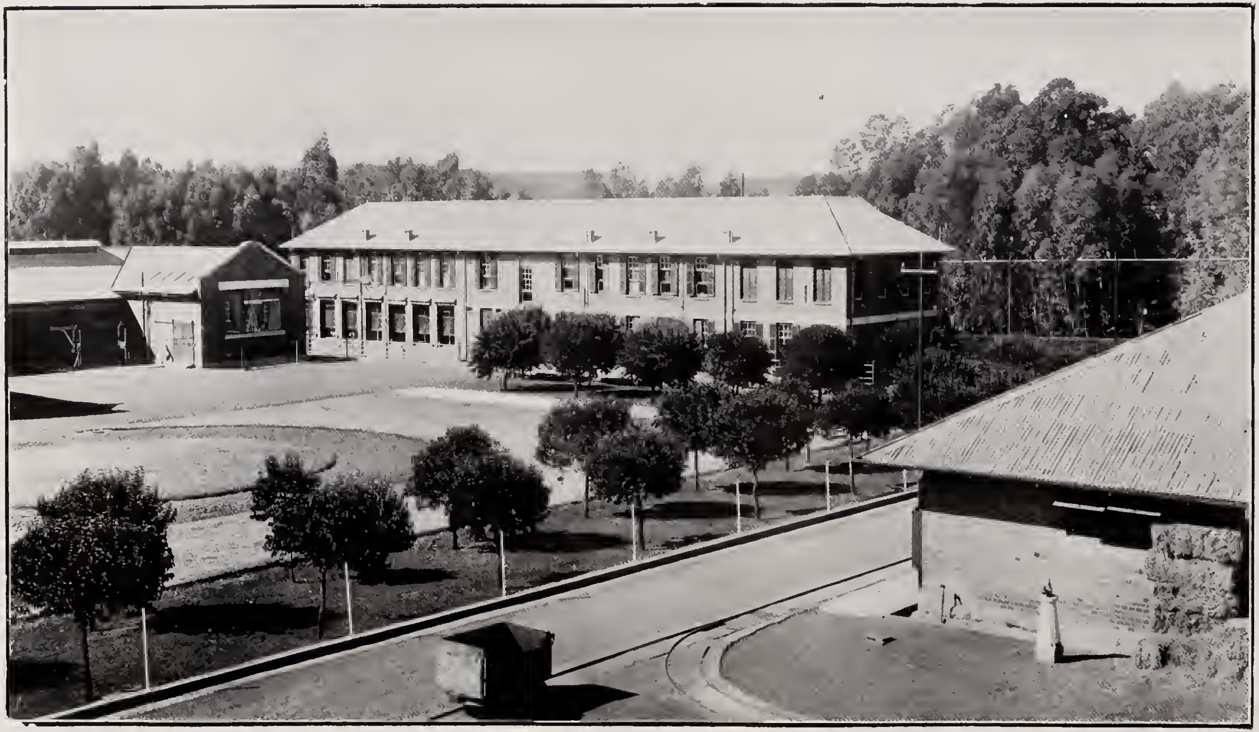
* Now out of print.

The " Main Building," Onderstepoort. Erected 1908.



Air-view of a portion of Onderstepoort, looking north-west, with the "Pyramid" hills in the background.



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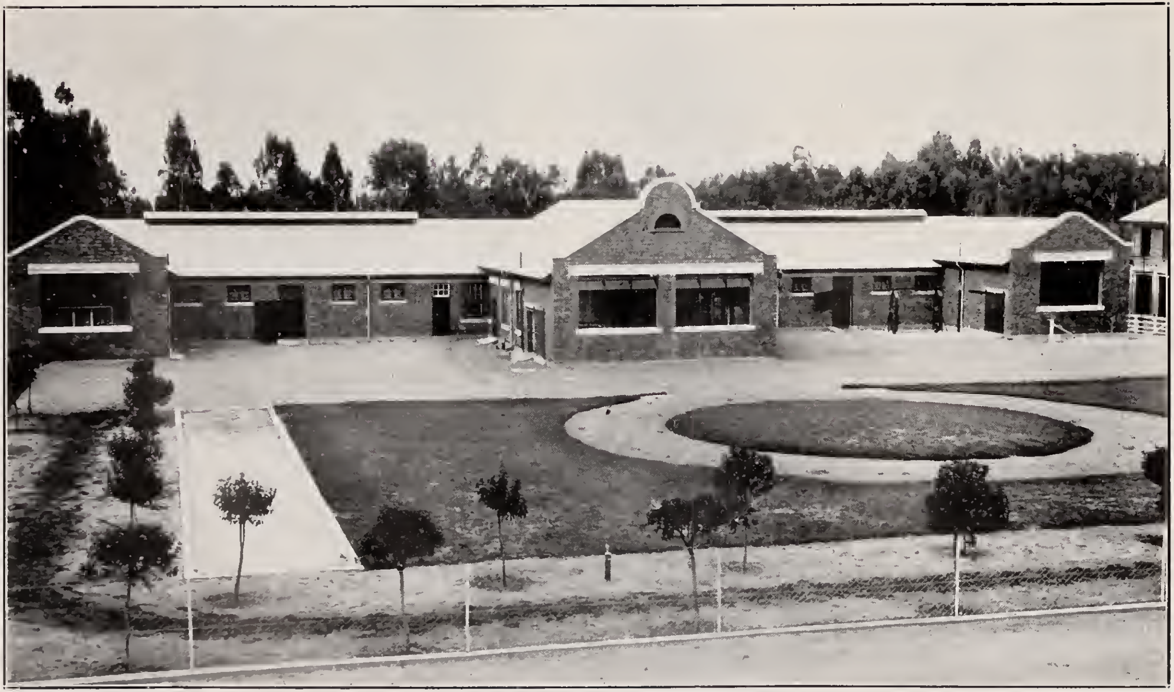

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Section I.

Protozoal Diseases.

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15th Annual Report of the Director of Veterinary Services, Union of South Africa. October, 1929.

A Note on the Diagnosis of *Trypanosoma vivax* Infection.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services.

One of the difficulties experienced by veterinarians working in the tsetse fly belt of the Union of South Africa has been the diagnosis of Nagana. When trypanosomes are found in the peripheral blood stream, the diagnosis is simple enough; but often cases are encountered in which the clinical symptoms point to Nagana but where no trypanosomes can be found in blood smears. Such a state of affairs is unsatisfactory, and any method which will assist in the diagnosis is to be welcomed.

Generally speaking, *Trypanosoma congolense* can be found much more easily in the blood of cattle than *Trypanosoma vivax*. The former may be very frequent, and even in chronic cases of infection is seldom entirely absent for a long period, whereas the latter is usually so rare that even a prolonged search will fail to reveal them in blood smears prepared in the ordinary way.

The difficulty of finding *T. vivax* in blood smears or the fresh blood preparations (hanging drop) is clearly demonstrated in an article by Curson (1928), in which details are given of experiments carried out by Berg in 1924.

In these experiments it was attempted to transmit *T. vivax* infection from two cattle into sheep, goats and horses. Fairly large quantities of fresh blood (20-50 c.c.) were used for the subinoculations. Altogether about 20 animals were injected in this way; but, although clinical symptoms which were attributed to the *T. vivax* infection, were observed in the majority of cases and some of them died, trypanosomes were seen only in one sheep on one occasion. In none of the other animals could the trypanosomes be demonstrated in the blood.

An interesting observation on the diagnosis of *T. vivax* in cattle was made at this Laboratory under the following circumstances:—

At Christmas, 1927, the late Dr. Claude Fuller, former Chief Entomologist for the Union of South Africa, returned from Zululand where he had been engaged on tsetse fly investigations on behalf of the Division of Veterinary Services, and brought some live specimens of *Glossina pallidipes* with him. These flies were fed daily on various animals, amongst others on heifer 1199. Feeding on this animal took place on the 30th and 31st January, 1928. Five weeks later a few trypanosomes of the type of *T. vivax* were found in the blood of

1199. The trypanosomes may have been present earlier, but as the flies were not suspected of being infected, the blood of 1199 was not examined regularly.

On the 8th March, 1928, a subinoculation was made from 1199 into heifer 2475. On the 9th, 10th and 11th days after inoculation trypanosomes were found in small numbers in the blood of 2475; but after the 11th day no further trypanosomes could be found in the blood smears.

On the 2nd April, 1928, 5 c.c. blood of heifer 2475 was subinoculated into calf 2583. Trypanosomes appeared on the 7th day after the injection and were found in small numbers on the 8th, 14th, 16th and 19th days. On the intervening days, and subsequent to the 19th day, no trypanosmes could be found in the blood smears. Apparently calves 2475 and 2583 had recovered spontaneously.

In July, 1928, a further attempt was made to recover this strain of trypanosomes. Calves 2475 and 2583 had meanwhile been used in other experiments in which they died. The only hope, therefore, was to recover the strain from 1199. Accordingly on the 14th July, 1928, 50 c.c. of blood was taken from 1199 and injected into calf 2611. A few trypanosomes were found in the blood of 2611 on the 8th day and again on the 27th and 28th days after the injection. These were the only occasions upon which *T. vivax* was seen in blood smears from 2611.

On the 8th August, 1928, 20 c.c. blood was subinoculated from 2611 into calf 2793. In spite of repeated examinations of blood smears, no trypanosomes could be found in this latter calf. On the 20th September, a further quantity of 50 c.c. blood was injected from 2611 into 2793, but again *T. vivax* failed to appear in the blood.

These subinoculations from 2611 into 2793, whilst apparently failing to transmit *T. vivax*, did succeed in setting up an infection of *Piroplasma bigeminum*, *Anaplasma marginale* and *Gonderia mutans* in 2793.

On the 19th October, 1928, while searching for possible developmental stages of *Gonderia mutans* in *gland* smears of 2793 *T. vivax was found in fairly large numbers.* A careful and prolonged examination of the blood smears of this animal failed to reveal any trypanosomes. The following days the same observation was made: *no trypanosomes found in the blood smears, but trypanosomes fairly frequent in gland smears.*

Thereupon calf 2611 was again examined. Trypanosomes had last been seen in rare numbers in the blood of this calf on the 10th August, 1928. Blood and gland smears were now prepared (20th October, 1928) with the result that no trypanosomes were found in the blood smears, whereas trypanosomes were present in the gland smears.

The value of gland examination for the diagnosis of T. vivax seemed obvious from these observations. To test the point further 6 calves (Nos. 2778, 2780, 2784, 2789, 2790 and 2800), which were under daily observation in another experiment, were injected each with 50 c.c. blood from 2793 on the 24th October, 1928. Blood and gland smears have been examined regularly since then. It is unnecessary to go into great detail; suffice it to say that trypanosomes were found with great regularity and frequently in large numbers in the gland smears, whereas they were only rarely seen in the blood smears.

Up to date (January, 1929) the number of blood and gland examinations of these calves is 68. On eight occasions no trypanosomes were found either in blood or gland smears; no less than 55 times were trypanosomes found in gland smears when no trypanosomes could be found in the blood smears, and only on five occasions were both blood and gland smears positive. Thus it will be seen that trypanosomes were found in blood smears only five times out of a total of 68 examinations (7.4 per cent.) whereas they were present in the gland smears 55 times (80.9 per cent.). In three animals (2778, 2789 and 2800) trypanosomes were never seen in the blood smears, so that a negative diagnosis would have been returned in these cases if the glands had never been examined.

The marked difference between blood and gland smears can be illustrated by a few examples:

Date: 2nd November, 1928.

Calf 2789, *blood smears*: no trypanosomes seen (10 minutes search);
gland smears: *T. vivax* very frequent (8 and more per field).

Calf 2784, *blood smear*: one specimen of *T. vivax* found after prolonged search;
gland smears: *T. vivax* very frequent (10 to 20 trypanosomes per field).

Figure 1 illustrates the frequency of the trypanosomes in the gland smear just referred to.

Entries like: "blood smear negative, gland smear *T. vivax* very frequent," occur throughout the experimental record.

The question arose whether the trypanosomes would be more frequent in some glands than in others. To test this an animal was selected (2778) on a day (31/11/1928) when trypanosomes were found in rare numbers in the gland smears, the blood smear being negative. Smears were then prepared from the right and left prescapular and the right and left precrural glands. In each of these four smears the trypanosomes were found to be rare, so that eight or ten specimens were seen during a five to ten minutes' search. There would thus seem to be no essential difference between the glands just mentioned.

On looking through the literature a reference was found to this peculiar predilection of *T. vivax* for the lymph glands in an article by H. E. Hornby, "Trypanosomes and Trypanosomiases of Cattle." *Jnl. of Comp. Path. and Therap.*, Vol. 34, Part 3, September, 1921, where the author writes (on page 227): "I have found that examination of gland juice is more likely to yield positive results than is examination of blood. A good routine procedure includes the examination of both fresh gland juice and a stained blood film."

The observations recorded above have shown that for the diagnosis of *T. vivax* in cattle, gland smears are very much more reliable than blood smears. In no single instance did a blood smear reveal trypanosomes when they were not also present (usually in much larger numbers) in the gland smear. But, on the other hand, the gland smears very frequently showed trypanosomes when the corresponding blood smears were negative (55 times out of 60 positive diagnoses).

The recommendation of Hornby may thus be amended to read, that *a good routine procedure also includes the examination of stained gland smears.* As a matter of fact, in our examinations it was easier to find trypanosomes in dried gland smears than in a preparation of fresh gland juice.

CONCLUSION.

Examination of stained gland smears has been found to be a valuable method of diagnosing *T. vivax* in cattle. The chances of diagnosing the infection in the gland smear as compared with the blood smear are as 12 to 1.

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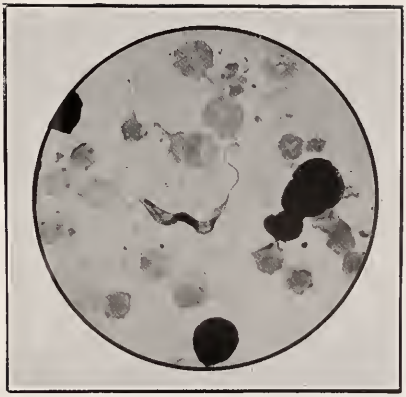
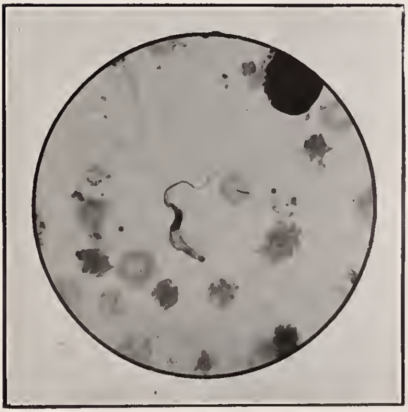
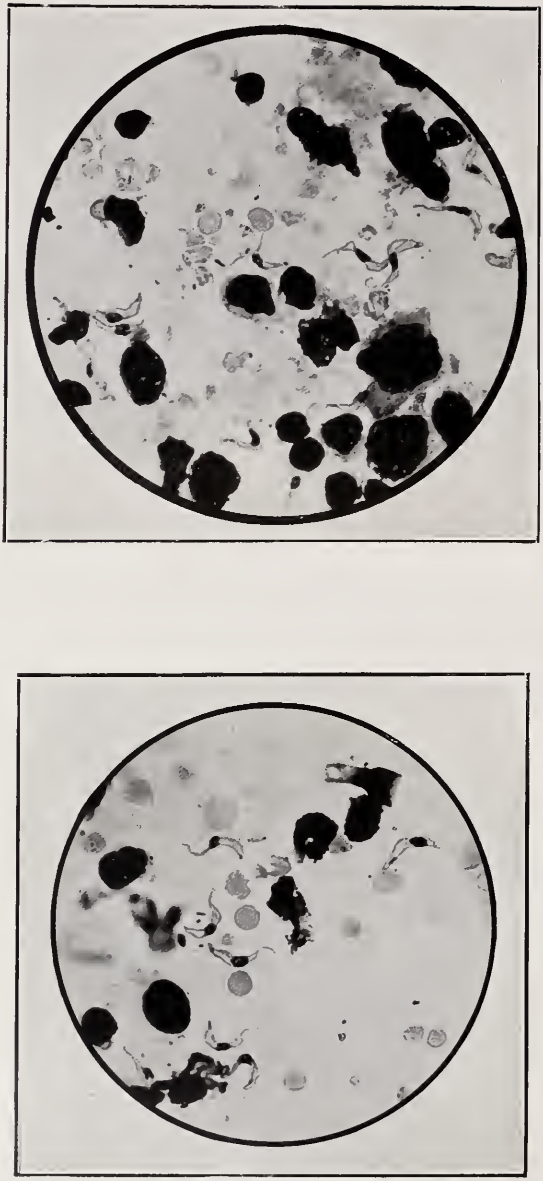




Figs. 1 and 2.—Single specimens of *Trypanosoma vivax*, as seen in the gland smears of Calf 2784.



FIGS. 3 AND 4.—Numerous specimens of *Trypanosoma vivax* in a gland smear of Calf 2784.

*15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.*

The Relation of the Spleen to Immunity in Bovine Piroplasmosis.

By G. DE KOCK, M.R.C.V.S., Dr.Med.Vet., D.Sc., Sub-Director of Veterinary Services.

De Kock and Quinlan (1926) showed that the course of ovine anaplasmosis in South Africa was of such a mild nature that it was not identified for a long time. It was only in 1924, when carriers of ovine *Anaplasma* were splenectomized, that the disease was recognized for the first time. Relapses of acute anaplasmosis followed, which in some instances proved fatal. In the same way splenectomy in equines, the carriers of *Nuttallia equi*, was followed by fatal relapses of nuttalliosis. In bovines relapses of anaplasmosis alone, or combined with piroplasmosis and gonderiosis, followed.

According to Ziemann (1924), it would appear that human patients without a spleen showed new malaria relapses, in some instances ending fatally. Dealing with splenectomy in apes, Gonder and Rodenwalt (1910) maintain that *Plasmodium kochi* usually disappears quickly from the blood, whereas after splenectomy they remain in the blood in large numbers for months. A similar observation was made by them in cases of splenectomy on dogs infected with *Piroplasma canis*.

Lauda (1925) showed that the extirpation of the spleen in rats was followed by a severe anaemia, and that this was due to some type of virus vegetating on the mucous membrane of the stomach or intestine, but held in abeyance by the spleen. When the spleen is removed, the virus gains entrance to the body, and after an incubation period attacks the red corpuscles. Lauda was able to reproduce the disease in splenectomized susceptible rats.

Lauda's work was confirmed by Mayer and his co-workers (1927), who noted that the corpuscles of splenectomized rats, affected with this severe anaemia, contained small bacilliform bodies (Bartonella), resembling *Bartonella bacilliformis*. Ford and Eliot (1928) found that the blood of anaemic splenectomized rats, taken three to six days after removal of the spleen, will produce anaemia in young normal rats with intact spleen, provided that these rats are not too old or too large. The disease develops in these young animals in much the same way that it does in adults after splenectomy. Regendanz and Kikuth (1928) showed that splenectomy in "marsupial rats" was followed by the appearance in the blood of a new piroplasm (*Nuttallia brasiliensis*). A latent spirochaetal infection also became activated by splenectomy. Kikuth (1928) found that dogs infected with *Piroplasma canis*, and *Bartonella canis*, when splenectomized, showed fatal relapses in eight cases out of nine. The reactions were so acute that therapeutic treatment (i.e. trypan-blue) had no effect on the reactions. Regendanz (1928) maintains that *Spirochaete didelphydes* multiplies so rapidly in the blood after splenectomy that death follows in a comparatively short while.

From the above it would therefore seem that the spleen has a distinct bearing on the course and immunity of some of the protozoal and bacterial diseases in man and animals. The exact nature of this function of the spleen is not understood. The object of the present paper is to record certain observations made in splenectomized bovines with regard to certain aspects of their immunity against piroplasmosis.

These observations were made at Onderstepoort in 1928 on bovines splenectomized in 1925 (see Table 1).

TABLE 1.

No. of Animal.	When Splenectomized.	Result.	Subsequent History.
1034......	20/1/25.......	10/2/25, relapse of piroplasmosis and anaplasmosis. It recovered from the the former.	On 23/6/28 injected with the local strain of piroplasma and anaplasma. Died of acute redwater on 5/7/28.
1032......	21/4/25.......	26/4/25, relapse of piroplasmosis and anaplasmosis. It recovered completely from the former.	On 10/2/28 turned on to the veld (west camp). On 20/3/28 died of acute redwater contracted naturally.

From the above table the following facts seem to be evident:—

(1) both animals were carriers of *Piroplasma* and *Anaplasma* before being splenectomized;

(2) in both, relapses of piroplasmosis and anaplasmosis occurred after splenectomy;

(3) both animals recovered from redwater relapses, and in the interval between the last reaction in 1925 and reinfection in 1928, no further reactions occurred while these animals were being stabled at Onderstepoort;

(4) in 1928, as a result of reinfection, they seemed to have lost their immunity, in case of the one animal after the injection of blood containing the local strain, and in the other case following natural infection locally.

Bovines reared on the veld at Onderstepoort or artificially immunized against piroplasmosis and anaplasmosis seem to acquire a lifelong immunity against the local strain. When immunized animals are turned on to the veld, they are able to withstand natural infection. In malaria, it is maintained that the following conditions may bring about a relapse, e.g. colds, excitations, exposure, sudden exertion, abnormal heat, traumatic influences, etc. It is said that breakdowns in the immunity of piroplasmosis may occur as the result of such debilitating diseases as East Coast fever and rinderpest. These latter two diseases can be definitely ruled out at Onderstepoort, and it is extremely doubtful whether there are any authentic cases on record there where breakdowns in immunity have occurred in immunized bovines, as a result of some other disease (e.g. heartwater). The above would thus indicate that the cattle at Onderstepoort, before being turned on to the veld, acquire a good lasting immunity against the local strain.

From the acute nature of the reinfection which took place in 1928, when the immunity of Bovines 1032 and 1034 (both local animals) were subjected to a test, it would appear that both animals had completely lost their immunity. In fact, they seemed to have become more susceptible than the ordinary locally bred animal. In both instances nearly all the erythrocytes in the blood and organs revealed the presence of parasites, and both animals showed the presence of haemo-lymphoid-like nodules in the liver. These nodules are fully described and discussed by De Kock (1929). How soon after splenectomy an animal loses its immunity cannot be determined from the above observations. This susceptibility in splenectomized bovines only manifested itself after the animals had been exposed to infection.

Locally bred animals naturally immunize more readily than imported cattle. This may probably be due to some inherited resistance. It is a well-known fact that young animals of any locality where piroplasmosis and anaplasmosis exist become naturally immunized to that local strain without manifesting severe clinical symptoms. On the other hand, it has been shown that, if regular and constant dipping is practised, this " inherited " resistance may actually become reduced, or even lost in the progeny, and such animals become almost as susceptible to redwater and gallsickness as imported bovines.

Recent observations made at Onderstepoort showed that splenectomized locally bred bovines (e.g. 870 and 894), when exposed to the local strain naturally, died of acute redwater. Both these animals after splenectomy in 1925 showed relapses of anaplasmosis and gonderiosis. They did not react to piroplasmosis, i.e. they were evidently still susceptible to that disease. Bovine 711, locally bred and splenectomized on 27.3.24 when it was only one year old, showed a slight relapse of anaplasmosis. On 6.3.25, when inoculated with the ordinary local strain of redwater and gallsickness, it showed a very acute reaction of redwater, to which it succumbed on 14.3.25.

When these results obtained in splenectomized locally bred cattle are compared with well-known facts about immunity in non-splenectomized animals, it would again appear that the spleen materially assists the animal in acquiring and maintaining its immunity. Splenectomy in the locally bred animal in some way or other interferes with the natural inherited resistance, referred to above in locally bred cattle. Such splenectomized animals, when exposed to the local natural infection, die of peracute redwater. Their susceptibility resembles that of imported cattle, and in fact becomes even more enhanced.

A similar observation was made by De Kock and Quinlan in case of anaplasmosis in sheep. Susceptible non-splenectomized sheep inoculated with *Anaplasma ovis* only revealed slight symptoms of an oligocythaemia without any clinical manifestations whatsoever. On the other hand, susceptible splenectomized sheep infected with *Anaplasma ovis* showed acute reactions of anaplasmosis , which actually proved fatal in some cases.

DISCUSSION.

From the above observations it would definitely appear that the spleen plays a part in the acquisition and maintenance of immunity in some of the protozoal diseases of man and animals. Unfortunately, the writer was handicapped in not being able to obtain more references of importance relating to the rôle played by the spleen in immunity. The few available may briefly be referred to. Knuth and Du Toit (1921) maintain that the immunity in piroplasmosis is more of the nature of a resistance (labile infection) than a real immunity, and this resistance is acquired and not inherited; the resistance of calves born on tick-infested areas is not hereditary, but acquired, etc. According to the observations of Gonder and Rodenwalt (1910), the spleen seems to have a decided controlling influence over infection in some of the protozoal diseases. Saigols, according to Gonder and Rodenwalt, encountered a fatal case of malaria in a human with congenital splenic aplasia. Ziemann is unable to suggest what exactly is the rôle of the spleen in these protozoal infections. Ford and Eliot (1928) are of opinion that these cases of anaemia in splenectomized rats which have once had the disease, and the spontaneous and induced relapses in recovered splenectomized rats, point to the failure of the immunizing mechanism in the absence of the spleen. Why the young spleen is unable to protect the animal against invasion with the virus, and the adult spleen is usually so effective, is, according to them, a matter for future investigation. Other organs do not assume this function of protection, shown by the relapse of splenectomized rats many months after recovery. Cl. Schilling (1927) maintains that labile infections depend on the balance between host and parasite, in which case antibodies are formed in such amounts that no marked increase of the parasites takes place. If this balance is upset by any injury to the host, then rapid multiplication of the parasites may take place. Regendanz (1928) refers to an observation of Tictin, viz., that immunity also takes place in splenectomized apes, and when such immunity is present, it does not disappear after splenectomy. Regendanz, according to his own observations on the occurrence of *Spirochaete didelphydes* in the splenectomized opossum, states that spirochaetes multiply so rapidly in the blood that death follows in a relatively short time. The antibodies in the case of the non-splenectomized opossum destroy the spirochaetes quickly and set up an immunity. He further discusses the question as to whether the spleen alone is the building centre of antibodies in case of spirochaetosis. Regendanz is of the opinion that the spleen plays an important, if not an exclusive, rôle in the formation of antibodies in case of spirochaetosis. According to the observations of Lannoy and Brühl (quoted by Regendanz), the spleen only plays a relative part in the formation of antibodies in fowl spirochaetosis. It has, however, been shown by many investigators that the functions of the reticulo-endothelial system as studied in mammals cannot be compared with that of birds.

In this brief discussion of the literature, it will be realized that very little exact information is available about the precise function of the spleen in bringing about and maintaining the immunity in such diseases as piroplasmosis, anaplasmosis, spirochaetosis and bartonella. Some authors are definitely of the opinion that the spleen is the only organ capable of setting up an immunity and that it plays the sole part in the formation of antibodies.

It would appear that these antibodies are continually liberated in the spleen by virtue of a labile or latent infection which is maintained by the spleen probably on account of its involved system of blood sinuses.

In the case of splenectomy in piroplasmosis and anaplasmosis, a relapse takes place as a result of the multiplication of the parasites present in the circulation. The antibodies still circulating in the system will determine whether the animal can recover from such a relapse or whether the parasites will predominate and bring about the death of the animal. If the animal recovers from such relapse, it may remain immune for some time by virtue of the antibodies still present in the circulation. The amount of antibodies present in the blood after splenectomy will determine the course of a relapse. Due to the absence of the spleen, no further antibodies are produced, and in this way the animal in time again becomes susceptible.

From the above observations it would appear that the spleen alone functions in the formation of antibodies. Bovines succumbed to a reinfection, in spite of the fact that they revealed at post-mortem hypertrophy of haemo-lymph glands and the presence of numerous haemo-lymphoid-like nodules in the liver. Stephens (1927) maintains that in the case of haemo-lymph glands analogy with the structure of the splenic pulp suggests similar functions for the spleen. The spleen also seems to play a part in respect of an inherited immunity in piroplasmosis. Most probably calves born from animals running on infected veld are much less susceptible than calves from imported animals. It was shown that when such calves of the locality are splenectomized they seem to lose this inherited resistance and become as it were hypersusceptible to infection. In the human being a fatal case of malaria is recorded in congenital splenic aplasia.

CONCLUSIONS.

(1) There seems to be no doubt that the spleen has a distinct bearing on the course and immunity of some of the protozoal diseases in man and animals. It is responsible for the acquisition and maintenance of immunity.

(2) The mechanism of this immunity as displayed by the spleen is not understood.

(3) Cattle at Onderstepoort acquire, either naturally or artificially, a good lasting immunity against the local strain of redwater and gallsickness.

(4) Splenectomized bovines, although bred at Onderstepoort, seem to become far more susceptible than the other animals with a spleen, when exposed to natural infection.

(5) Locally bred animals immunize naturally more easily than imported stock. This is probably due to some inherited resistance.

(6) Splenectomy practised in the locally bred animal seems to interfere with natural inherited resistance.

(7) It would appear that immunity results from the production of antibodies by the spleen.

(8) The spleen seems to be the only organ capable of maintaining the production of antibodies.

(9) The amount of antibodies present in the circulation will determine whether the animal survives the relapse following splenectomy or not.

(10) The animal after recovery from a relapse may remain immune as long as there are antibodies present in the circulation. This immunity is temporary and disappears completely after some time, probably due to the fact that no spleen is present to produce further antibodies.

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The Transmission of East Coast Fever by Means of Blood.*

By Sir ARNOLD THEILER, K.C.M.G., D.Sc., etc., late Director of Veterinary Education and Research,

and

P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services, Onderstepoort.

In 1927 Sergent, Donatien, Parrot, Lestoquard and Plantureux published an article on the Theilerioses of cattle, with special reference to " East Coast fever " and the " North African theileriosis." In this article experiments are described which were carried out with ticks infected with *Theileria parva*, which were obtained from the Onderstepoort Laboratories. Sergent kindly sent us a summary of the results before the publication of the article.

The fact that these authors had succeeded in transmitting East Coast fever by means of blood inoculations came as a great surprise to us; for, up till then, it was always believed that this disease could not be transmitted with the blood of infected animals. This conclusion was reached after many unsuccessful injections of blood into the jugular vein. We had also carried out inoculations under the skin and into the glands, with negative results, but probably the number of inoculations had not been large enough to warrant a final conclusion.

After receipt of Sergent's report we decided therefore to repeat, with South African cattle, the experiments carried out by the North African workers. The following list indicates the method of injection employed and the results obtained by them:—

A. Intraperitoneal: one calf, result negative.
B. Subcutaneous, intradermal and into the glands: one calf, result positive.
C. Intrajugular: two calves, result negative.
D. Subcutaneous: one calf, result negative.
E. Into the glands: one calf, result negative.
F. Intradermal: one calf, result negative.
G. Subcutaneous, intraperitoneal, intradermal and into the glands: two calves, one positive and one negative.
H. Intraperitoneal and intradermal: one calf, result negative.
I. Subcutaneous and intradermal: two calves, one positive and one negative.

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* This article has appeared, in a slightly different form, in French, in the " Bulletin de la Société de Pathologie exotique," Vol. 21, No. 2, pp. 121-132, February, 1928.

Among the twelve inoculated calves there were thus only three positive cases, and all of these had been injected in two to four different places.

Since it was thought that the North African cattle might possibly be more susceptible than our South African cattle, we decided to repeat the experiments, using the same quantity of blood, but taking a larger number of animals in order to determine which method would yield the best results.

We shall begin with the history of the animals from which the blood was obtained for the inoculations and shall then describe the experiments in detail.

BLOOD DONORS FOR THE INOCULATIONS.

I. Natural Infections.

1. *Heifer* 1195 (Experiment 2890). Infected nymphae of *Rhipicephalus appendiculatus* (Batch 782) were fed on this heifer on the 3rd January, 1927. The incubation period lasted 11 days, the fever reaction 19 days; the heifer died of East Coast fever. Koch's bodies were found in the glands and small piroplasms (*Theileria parva*) were present in large numbers in the blood, which was subsequently used to inject the following cattle:—

2096, 1199, 2038 and 1944 (in experiment 2918); 1114, 1671 and 2207 (in experiment 2951); 1776, 2054, 1894 and 1196 (in experiment 2920); 2076, 1803, 2066 and 1656 (in experiment 2919).

2. *Ox* 1944 (Experiment 3008) was infected on the 18th March, 1927, with nymphae of *Rhipicephalus appendiculatus* (Batch 856). The incubation period lasted 12 days and the fever reaction 13 days, after which the animal died of East Coast fever. Koch's bodies were found in the glands, and very numerous small piroplasms (*Theileria parva*), and also Koch's bodies in the blood. The blood was used for injection into the following cattle: 2230, 2192, 2102, 2104, 2107 and 2108 (experiment 3041).

3. *Ox* 2096 (Experiment 3008) was infected on the 14th April, 1927, with adults of *Rhipicephalus appendiculatus* (Batch 857). It contracted and died of East Coast fever. The incubation lasted 10 days, the febrile period 16 days. Koch's bodies were found in the glands on the third day of the fever, and small piroplasms (*Theileria parva*) were present in large numbers in the blood towards the end of the disease. The blood of ox 2096 (mixed with the blood of 2119 and 2192) was used for the injection of cattle 2122 and 2193 (experiment 3057).

4. *Ox* 1109 (Experiment 2835) was infected on the 20th November, 1926, with adults of *Rhipicephalus appendiculatus* (Batch 848). It contracted East Coast fever and recovered. The incubation period lasted nine days and the fever reaction 17 days. Small piroplasms (*Theileria parva*) were present in the blood, which was used for the infection of cow 1477 (experiment 2872).

5. *Bull* 1015 (Experiment 2836) was infected on the 20th November, 1926, with nymphae of *Rhipicephalus appendiculatus* (Batch 781), as a result of which it contracted East Coast fever and died.

The incubation period lasted nine days, the fever reaction 23 days. A large number of Koch's bodies were present in the glands, and small piroplasms (*Theileria parva*) were very numerous in the blood, which was used for the infection of cow 1486 (experiment 2872).

6. *Ox* 1045 (Experiment 2836) was infected on the 20th November, 1926, with nymphae of *Rhipicephalus appendiculatus* (Batch 781); it contracted East Coast fever and died. The incubation period lasted nine days and the fever reaction 14 days. Koch's bodies were present in large numbers in the glands and small piroplasms (*Theileria parva*) were very numerous in the blood, which was used for the infection of heifer 1484 (experiment 2872).

7. *Heifer* 2452 (Experiment 3070) was infected on the 10th June, 1927, with nymphae of *Rhipicephalus appendiculatus* (Batch 868). It contracted East Coast fever and died. The incubation period lasted 11 days and the fever reaction 20 days. Koch's bodies were present in the glands and small piroplasms (*Theileria parva*) were very numerous in the blood, which was used for the injection of cattle 2102, 2104 and 2193 (experiment 3057.)

8. *Heifer* 2408 (Experiment 3032) was infected on the 16th July, 1927, with nymphae of *Rhipicephalus appendiculatus* (Batch 868). She contracted East Coast fever and died. The incubatoin period lasted 10 days and the fever reaction 20 days. Koch's bodies were found in the glands on the sixth day of the reaction and small piroplasms (*Theileria parva*) were present in large numbers in the blood, which was used for the inoculation of cattle 2102 and 2104 (experiment 3185).

II. Infection by Means of Blood Inoculations.

9. *Heifer* 1656 (Experiment 2919) received on the 30th January, 1927, injections of 165 c.c. blood of heifer 1195 (Donor No. 1) under the skin, into the peritoneal cavity, into the glands, and into the skin. She contracted East Coast fever and died. The incubation period lasted 15 days and the fever reaction 11 days. Koch's bodies were found from the fourth day of the reaction onward in the glands, and small piroplasms (*Theileria parva*) were numerous in the blood, which was used for the injection of cattle 1773, 1894, 2054 and 2076 (experiment 2990), and 2098 (experiment 2989).

10. *Tollie* 2038 (Experiment 2918) received on the 20th January, 1927, injections of 252.5 c.c. of blood of heifer 1195 (Donor No. 1) subcutaneously and intradermally, as a result of which it contracted East Coast fever and died. The incubation period lasted 12 days and the fever reaction 26 days. Koch's bodies appeared in the glands on the second day of the reaction and small piroplasms (*Theileria parva*) were very numerous in the blood, which was used for the inoculation of cattle 2104, 1759 and 2119 (experiment 2961).

11. *Bull* 1894 (Experiment 2990) received on the 24th February, 1927, injections of 252.5 c.c. blood of heifer 1656 (Donor No. 9) subcutaneously and intradermally; this case represented the second generation of successful transmissions of East Coast fever by means of blood inoculation. The incubation period lasted 18 days and the fever reaction 10 days, after which the animal recovered. Koch's bodies were found in the glands from the second day onward, and

small piroplasms (*Theileria parva*) were present in large numbers in the blood, which was used for the injection of cattle 1477, 1725, 1759 and 2119 (experiment 3021); 2066, 1589 and 1803 (experiment 2989).

12. *Heifer* 2192 (experiment 3041) received on the 12th April, 1927, injections of 252.5 c.c. blood of ox 1944 (Donor No. 2) subcutaneously and intradermally and contracted East Coast fever. The incubation period lasted 13 days and the fever reaction 12 days when the animal was killed. Koch's bodies were present in large numbers in the glands, and small piroplasms (*Theileria parva*) were frequent in the blood, which was used for the inoculation of cattle 2122 and 2193 (experiment 3057); and 1477, 1725 and 1759 (experiment 3064).

13. *Heifer* 2119 (Experiment 3021) received on the 26th March, 1927, an intradermal injection of 5 c.c. blood of bull 1894 (Donor No. 11); this case therefore represented the third generation of successful transmissions of East Coast fever by means of blood inoculation. The incubation period lasted very long, namely, 28 days, and the fever reaction 17 days, when the animal died of East Coast fever. Koch's bodies were found in the glands and small piroplasms (*Theileria parva*) were numerous in the blood, which was used for the inoculation of cattle 2122 and 2193 (experiment 3057).

14. *Tollie* 2122 (Experiment 3057) received on the 5th May, 1927, 100 c.c. defibrinated blood of cattle 2096 (Donor No. 3), 2119 (No. 13), and 2192 (No. 12) into the peritoneal cavity. It contracted East Coast fever and died. The incubation period lasted 15 days and the fever reaction 20 days. Koch's bodies were present in the glands and small piroplasms (*Theileria parva*) were numerous in the blood, which was used for the inoculation of cattle 1725 and 1759 (experiment 3064).

III. Infection by Means of Injection of an Emulsion of Infected Ticks.

15. *Heifer* 1121 (Experiment 2484) received on the 12th April, 1926, an injection of an emulsion of 12 nymphae of *Rhipicephalus appendiculatus* (Batch 725). The incubation period lasted nine days and the fever reaction 20 days, when the animal died of East Coast fever. Koch's bodies were present in the glands and small piroplasms (*Theileria parva*) were numerous in the blood, which was used for the inoculation of cattle 1109 and 1114 (experiment 2525).

TRANSMISSION EXPERIMENTS.

A. Attempt to Transmit East Coast Fever by the Intrajugular Injection of Blood.

EXPERIMENT 2872.

1. *Cow* 1477 received on the 18th December, 1926, a transfusion of 1,000 c.c. blood of ox 1109 (Donor No. 4), on the 19th day of the fever reaction.

Result: negative.

2. *Cow* 1484 received on the 18th December, 1926, a transfusion of 1,000 c.c. blood of ox 1045 (Donor No. 6), on the 14th day of the fever reaction.
Result: negative.

This cow showed a rise of temperature on the 18th day after the transfusion, with small piroplasms in the blood.

3. *Cow* 1486 received on the 21st December, 1926, a transfusion of 1,000 c.c. blood of bull 1015 (Donor No. 5), on the 22nd day of the fever reaction.
Result: negative.

EXPERIMENT 2961.

4. *Ox* 1759 received on the 7th February, 1927, an injection of 100 c.c. blood of tollie 2038 (Donor No. 10), on the sixth day of the fever reaction.
Result: negative.

EXPERIMENT 2525.

5. *Ox* 1109 and

6. *Ox* 1114 received on the 10th May, 1926, a transfusion each of 2,000 c.c. blood of heifer 1121 (Donor No. 15), on the 20th day of the fever reaction.

Result: negative.

Ox 1109 had a rise of temperature on the first day and ox 1114 on the second day after transfusion; but after that the temperature was normal.

Summary.

Five cattle received transfusions of 1,000 or 2,000 c.c. blood and a sixth animal an injection of 100 c.c. blood of East Coast fever animals into their jugular vein without contracting the disease.

B. Subcutaneous Injection of Blood.

EXPERIMENT 2961.

1. *Heifer* 2119 received on the 7th February, 1927, a subcutaneous injection of 100 c.c. blood of tollie 2038 (Donor No. 10), on the sixth day of the fever reaction.
Result: negative.

EXPERIMENT 2989.

2. *Tollie* 2098 received on the 24th February, 1927, a subcutaneous injection of 250 c.c. blood of heifer 1656 (Donor No. 9), on the 10th day of the fever reaction.
Result: positive.

This animal showed an irregular fever reaction from the sixth to the 18th day after injection, with *Piroplasma bigeminum* in the blood. Koch's bodies were present in small numbers in the glands, and small piroplasms (*Theileria parva*) were seen in the blood after the fever reaction.

The immunity of this animal was tested on no less than seven occasions with ticks, nymphae and adults of *Rhipicephalus appendiculatus*, which had transmitted East Coast fever to other animals, but without result; on two further occasions infected blood was injected into this tollie, but again with negative results. The immunity of tollie 2098 was therefore beyond doubt.

3. *Ox* 2066 received on the 25th March, 1927, a subcutaneous injection of 250 c.c. blood of bull 1894 (Donor No. 11), on the 11th day of the fever reaction.
Result: negative.

4. *Heifer* 1589 and

5. *Ox* 1803 received on the 25th March, 1927, a subcutaneous injection of 250 c.c. each of blood of bull 1894 (Donor No. 11), on the 11th day of the fever reaction.
Results: negative.

These animals received on the 5th May, 1927, each 2,000 c.c. blood subcutaneously of Donors Nos. 2096 (3), 2119 (13), and 2192 (12).
Results: again negative.

EXPERIMENT 3041.

6. *Ox* 2107 and

7. *Heifer* 2108 received on the 12th April, 1927, each a subcutaneous injection of 250 c.c. blood of ox 1944 (Donor No. 2), on the 12th day of the fever reaction.
Results: negative.

These two animals showed an irregular temperature with *P. bigeminum*, *Anaplasma marginale* and small piroplasms in the blood.

Summary.

Seven cattle received subcutaneous injections of 100 to 2,000 c.c. of East Coast fever blood, with one positive and six negative results.

C. INTRADERMAL INJECTION OF BLOOD.

EXPERIMENT 2961.

1. *Tollie* 2104 received on the 7th February, 1927, an intradermal injection of 10 c.c. blood of tollie 2038 (Donor No. 10), on the seventh day of the fever reaction.
Result: negative.

EXPERIMENT 3021.

2. *Cow* 1477;

3. *Ox* 1725;

4. *Ox* 1759; and

5. *Heifer* 2119 each received on the 26th March, 1927, an intradermal injection of 5 c.c. blood of bull 1894 (Donor No. 11), on the 12th day of the fever reaction.
Results: 1477, 1725, and 1759 *negative*, 2119 *positive*.

This heifer showed a fever reaction from the 29th day after injection until the 45th day, when it died. Koch's bodies were found in the glands and large numbers of small piroplasms (*Theileria parva*) in the blood.

EXPERIMENT 3041.

6. *Heifer* 2102 and

7. *Ox* 2104 each received on the 12th April, 1927, an intradermal injection of 5 c.c. blood of ox 1944 (Donor No. 2), on the 12th day of the fever reaction.
Results: negative.

Heifer 2102 showed an irregular temperature, which could not be regarded as East Coast fever. *Piroplasma bigeminum* and small piroplasms were found in the blood. The animal recovered.

Ox 2104 did not show a temperature reaction, but *Anaplasma marginale* and small piroplasms were seen in its blood.

EXPERIMENT 2951.

8. *Ox* 1114;

9. *Ox* 1671; and

10. *Tollie* 2207 each received on the 2nd February, 1927, an intradermal injection of 2.5 c.c. blood of heifer 1195 (Donor No. 1), on the 19th day of the fever reaction.
Results: negative.

Summary.

Nine cattle received intradermal injections of 10, 5 or 2.5 c.c. virulent blood. (Ox 2104 received two injections of 10 c.c. and 5 c.c.) Only one (heifer 2119) gave a reaction which could be interpreted as East Coast fever and ended fatally.

D. Intraperitoneal Injection of Blood.

EXPERIMENT 3057.

1. *Tollie* 2122 received on the 5th May, 1927, an intraperitoneal injection of 100 c.c. of a mixture of blood of ox 2096 (Donor No. 3), on the 9th day of the fever reaction, heifer 2192 (Donor No. 12) on the 10th day of the fever reaction, and heifer 2119 (Donor No. 13) on the 11th day of the reaction.
Result: positive.

This animal showed a fever reaction from the 16th day after the injection up to the day of its death, on the 35th day. In the glands numerous Koch's bodies were present, and in the blood both *Anaplasma marginale* and small piroplasms (*Theileria parva*) were found.

2. *Tollie* 2193 was treated in the same way as tollie 2122.
Result: positive.

The animal showed a fever reaction from the 19th to the 26th day after inoculation and recovered. *Anaplasma marginale*, *Piroplasma bigeminum* and small piroplasms were present in the blood, but Koch's bodies were not found in the glands.

This animal subsequently received two more intraperitoneal injections: on the 8th June, 1927, 100 c.c. blood of tollie 2122 (see above, this same experiment) on the 19th day of the reaction, and on the 7th July, 1927, 100 c.c. blood of heifer 2452 (Donor No. 7) on the 17th day of the fever reaction.
Result: negative.

The immunity of this animal was tested nine times with infected ticks, nymphae and adults of *Rhipicephalus appendiculatus*, which had produced cases of East Coast fever in other animals, but without results. It would seem more than probable therefore that the reaction which tollie 2193 showed after the intraperitoneal injeciton was actually a mild attack of East Coast fever; although this cannot be proved, seeing that Koch's bodies were never seen.

3. *Heifer* 2102 and

4. *Ox* 2104 each received on the 7th July, 1927, an intraperitoneal injection of 100 c.c. blood of heifer 2452 (Donor No. 7), on the 17th day of the fever reaction.

Results: negative.

Heifer 2102 had a rise of temperature on the 29th day after injection with small piroplasms in its blood, but this could not be regarded as an attack of East Coast fever.

Ox 2104 showed no reaction.

Summary.

Four cattle received intraperitoneal injections of virulent blood. Tollie 2122 contracted East Coast fever and died; tollie 2193 showed a doubtful reaction which may have been a mild attack of East Coast fever; and the other two remained negative.

E. Subcutaneous and Intradermal Injections of Blood.

EXPERIMENT 2918.

1. *Tollie* 2038 received on the 20th January, 1927, a subcutaneous injection of 250 c.c. and an intradermal injection of 2.5 c.c. blood of heifer 1195 (Donor No. 1), on the 6th day of the fever reaction.

Result: positive.

This animal showed an incubation period of 10 days and a fever reaction lasting 15 days, and died of East Coast fever. In the glands Koch's bodies were found, and in the blood small piroplasms (*Theileria parva*) were frequent, and *Piroplasma bigeminum* was also present.

2. *Ox* 1944 received on the 23rd January, 1927, the same quantities of blood as tollie 2038, above, taken from heifer 1195 (Donor No. 1), on the 9th day of the fever reaction.

Result: negative.

This animal showed no temperature reaction and was tested afterwards with infected ticks (nymphae of *Rhipicephalus appendiculatus*, batch 856), when it contracted East Coast fever and died (see Donor No. 2).

3. *Ox* 2096 received on the 27th January, 1927, a subcutaneous injection of 250 c.c. and an intradermal injection of 2.5 c.c. blood of heifer 1195 (Donor No. 1) on the 13th day of the fever reaction.

Result: negative.

This animal showed a slight temperature reaction from the 6th to the 10th day after injection, with small piroplasms in the blood. It was tested later on for immunity with infected ticks and died of East Coast fever (see Donor No. 3).

4. *Heifer* 1199 received on the 30th January, 1927, similar injections to the above of blood taken from heifer 1195 (Donor No. 1), on the 16th day of the fever reaction.

Result: negative.

This heifer had a fever reaction lasting six days, after an incubation of 14 days. Numerous Koch's bodies were found in the glands and large numbers of small piroplasms (*Theileria parva*) in the blood. This was, therefore, a case of East Coast fever with recovery.

EXPERIMENT 2990.

5. *Tollie* 1773;

6. *Bull* 1894;

7. *Tollie* 2054; and

8. *Tollie* 2076 each received on the 24th February, 1927, a subcutaneous injection of 250 c.c. and an intradermal injection of 2.5 c.c. blood of heifer 1656 (Donor No. 9), on the 10th day of the fever reaction.

Result: *positive* for all four.

Tollie 1773 had a reaction with an incubation of 13 days and a fever lasting 14 days, at the end of which it died. In the glands there were many Koch's bodies and in the blood small piroplasms (*Theileria parva*) and *P. bigeminum* were present.

Bull 1894 had a fever reaction lasting nine days with an incubation of 16 days and recovered. In its blood small piroplasms (*Theileria parva*) were found and in the glands large numbers of Koch's bodies.

Tollie 2054 showed a fever reaction of 12 days after an incubation of 14 days, and recovered. In its blood small piroplasms (*Theileria parva*) were found and in its glands Koch's bodies.

On the 30th April, 1927, infected ticks (adults of *Rhipicephalus appendiculatus*) (Batch 857), which transmitted East Coast fever to ox 2096 (Donor No. 3), were fed on tollie 2054 without producing a reaction, thus proving the immunity of this animal against East Coast fever.

Tollie 2076 had a slight reaction from the 12th to the 19th day after inoculation, with small piroplasms in the blood.

On the 4th April, 1927, and again on the 25th April, 23rd May, 14th July, 21st July, 26th July, and 3rd August infected ticks were placed on tollie 2076 without producing a reaction. On the 31st August injections of virulent blood were made subcutaneously, intradermally and intraperitoneally; and on the 28th September infected spleen and gland pulp was injected without breaking the immunity of this animal.

EXPERIMENT 3041.

9. *Heifer* 2230 and

10. *Heifer* 2192 each received on the 12th April, 1927, a subcutaneous injection of 100 c.c. (250 c.c. in the case of 2192) and an intradermal injection of 2.5 c.c. blood of ox 1944 (Donor No. 2), on the 12th day of the fever reaction.

Result: *negative* in the case of 2230 and *positive* in the case of 2192.

Heifer 2230 showed an irregular temperature with *P. bigeminum*, *A. marginale* and a few small piroplasms in the blood, and recovered.

Heifer 2192 showed a typical East Coast fever reaction with an incubation period of 13 days and a fever lasting 12 days, when the animal was killed. Koch's bodies were frequent in the glands and numerous small piroplasms (*Theileria parva*) and *P. bigeminum* were present in the blood.

Summary.

Ten animals received subcutaneous and intradermal injections. Three donors were used for these injections; of these heifer 1195 (No. 1) and ox 1944 (No. 2) had been infected by means of ticks, and heifer 1656 (No. 9) by means of blood inoculation. Two cases which were treated with the blood of 1195 (taken on the 9th and 12th day) were negative, and the other two cases were positive (blood taken on the 6th and 15th day). The animal injected with blood taken on the sixth day died, and the other, treated with blood on the 15th day, recovered.

Of the two animals injected with the blood of ox 1944 taken on the 12th day of the reaction, one was negative and the other contracted typical East Coast fever and was killed.

The four cattle injected with the blood of 1656, taken on the 10th day of the fever reaction, all contracted East Coast fever; one died and three recovered.

Hence of the 10 cattle used in this experiment, seven were positive (two deaths, one killed and four recoveries) and three were negative.

F. Injections of Blood Subcutaneously, Intradermally and into the Glands.

EXPERIMENT 2920.

1. *Tollie* 1776 received on the 20th January, 1927, a subcutaneous injection of 250 c.c., an intradermal injection of 2 c.c., and an injection into the glands of 10 c.c. blood of heifer 1195 (Donor No. 1), on the sixth day of the fever reaction.

Result: negative.

2. *Tollie* 2054 received on the 23rd January, 1927, the same injections as in the previous case of blood taken from heifer 1195 on the ninth day of the fever reaction.

Result: negative.

3. *Bull* 1894 received on the 27th January, 1927, injections as above, of blood of heifer 1195, taken on the 13th day of the fever reaction.

Result: negative.

4. *Heifer* 1196 received on the 30th January, 1927, injections as above, of blood of heifer 1195, taken on the 16th day of the fever reaction.

Result: doubtful.

This heifer showed a fever reaction from the 20th to the 24th day after injection with small piroplasms in the blood. No Koch's bodies were found in the glands, so that it seems unlikely that this animal reacted to the original injections.

Summary.

Three of the four cattle injected with blood of heifer 1195 on the 6th, 9th, and 13th day of the reaction remained negative. The fourth animal (heifer 1196) injected with blood taken on the 16th day showed a slight reaction which, however, was probably due to "*Gonderia*" *mutans* and not to *Theileria parva*. It is interesting to note that heifer 1656 in experiment 2919 (see below), which received blood from heifer 1195 on the same day, contracted East Coast fever.

G. Injections of Blood Subcutaneously, Intradermally, Intraperitoneally and into the Glands.

EXPERIMENT 2919.

1. *Tollie* 2076 received on the 20th January, 1927, a subcutaneous injection of 60 c.c., an intradermal injection of 5 c.c., an intraperitoneal injection of 60 c.c., and an injection into the glands of 40 c.c. blood of heifer 1195 (Donor No. 1), on the sixth day of the fever reaction.
Result: negative.

2. *Ox* 1803 received injections as above of blood of heifer 1195 on the 23rd January, 1927, the ninth day of the fever reaction.
Result: negative.

3. *Ox* 2066 received injections as above, of blood of heifer 1195, on the 27th January, 1927, the 13th day of the fever reaction.
Result: negative.

4. *Heifer* 1656 received injections as above, of blood of heifer 1195, on the 30th January, 1927, the 16th day of the fever reaction.
Result: positive.

This heifer died of East Coast fever. The incubation period lasted 15 days and the fever reaction 11 days. Koch's bodies were found in the glands and small piroplasms (*Theileria parva*) were numerous in the blood.

Summary.

Heifer 1195, which served as blood donor for this experiment, was suffering from East Coast fever for 19 days. Blood taken on the 6th, 9th, and 13th days of the fever reaction did not produce the disease in the animals which were injected; but blood taken on the 16th day (three days before death) produced the disease in heifer 1656.

H. Subcutaneous Injection of Blood and Rubbing Blood into Scarified Skin.

EXPERIMENT 3185.

In the experiments recorded in Section E above (experiments 2918, 2990 and 3041) it was shown that East Coast fever could be transmitted in a fairly high percentage of cases by means of subcutaneous and intradermal injections of blood. The present experiment was undertaken to determine whether the same results would be obtained if the blood was *rubbed* into the skin, instead of being injected into it.

1. *Heifer* 2102 and

2. *Ox* 2104 received on the 13th August, 1927, a subcutaneous injection of 25 c.c. blood of heifer 2408 (Donor No. 8), on the 17th day of the fever reaction, and at the same time blood was rubbed into the scarified skin at the seat of the injection.
Result: negative.

Summary.

Two cattle which received virulent blood subcutaneously and had the same blood rubbed into scarifications on the skin remained negative.

I. Rubbing Spleen and Gland Pulp and Blood into Scarified Skin.

EXPERIMENT 3064.

This experiment was undertaken to see whether the scarified skin is a suitable portal of entry for the East Coast fever infection. Spleen and gland pulp was used because this material is capable of setting up the infection fairly easily when injected into the jugular vein.

1. *Cow* 1477 was scarified on the back on the 7th May, 1927, and spleen and gland pulp obtained from heifer 2192 (Donor No. 12) was rubbed into the scarifications.
Result: negative.

Fresh scarifications were made on the 10th June, 1927, and infected spleen and gland pulp obtained from tollie 2122 (Donor No. 14) again rubbed in, with negative results.

2. *Ox* 1725 and

3. *Ox* 1759 were scarified on the 7th May, 1927, and treated with the same material as cow 1477. On the 8th June and again on the 9th June, 1927, fresh scarifications were made on their backs and infected blood of tollie 2122 (Donor No. 14) rubbed in.
Result: negative.

Summary.

Three animals were scarified on their backs and infected material (spleen and gland pulp and blood) rubbed into the scarifications with negative results.

In order to facilitate reference the results of the above experiments have been tabulated in the following way:—

No.	Method of Inoculation.	Expt. No.	Cattle No.	Quantity of Blood Injected.	Obtained from Donor No.	Date.	Result.		Immunity Test.			
							Transmission of East Coast Fever.	Fate of Animal.	Expt. No.	Date.	Ticks used.	Result.
A	Intrajugular......	2872	1477	1,000 c.c.	4	18.12.26	Negative	—	—	—	—	—
			1484	1,000 c.c.	6	18.12.26	Negative	—	—	—	—	—
			1486	1,000 c.c.	5	18.12.26	Negative	—	—	—	—	—
		2961	1759	100 c.c.	10	7.2.27	Negative	—	—	—	—	—
		2525	1109	2,000 c.c.	15	10.5.26	Negative	—	—	—	—	—
			1114	2,000 c.c.	15	10.5.26	Negative	—	—	—	—	—
B	Subcutaneous....	2961	2119	100 c.c.	10	7.2.27	Negative	—	—	—	—	—
		2989	2098	250 c.c.	9	24.2.27	*Positive*	Recovery	3032	3.4.27	*Rhip. append.* nymphae (batch 856)	Negative.
										25.4.27	*Rh. app.* adults (857) and nymphae (847)	Negative.
										23.5.27	*Rh. app.* adults (857)..	Negative.
										14.7.27	*Rh. app.* nymphae (868)	Negative.
										21.7.27	*Rh. app.* nymphae (868)	Negative.
										26.7.27	*Rh. app.* nymphae (869)	Negative.
										3.8.27	*Rh. app.* nymphae (889)	Negative.
										31.8.27	Subc., intradermal and intraperitoneal injections of E.C.F. blood	Negative.
										28.9.27	Subc., intrad., intraperit. and injection into glands of E.C.F. blood	Negative.
			2066	250 c.c.	11	25.3.27	Negative	—	—	—	—	—
			1589	(1) 250 c.c.	(1) 11	(1) 25.3.27	Negative	—	—	—	—	—
			1803	(2)2,000 c.c.	(2) 3, 12, 13	(2) 5.5.27	Negative	—	—	—	—	—
		3041	2107	250 c.c.	2	12.4.27	Negative	—	—	—	—	—
			2108	250 c.c.	2	12.4.27	Negative	—	—	—	—	—
C	Intradermal......	2961	2104	10 c.c.	10	7.2.27	Negative	—	—	—	—	—
		3021	1477	5 c.c.	11	26.3.27	Negative	—	—	—	—	—
			1725	5 c.c.	11	26.3.27	Negative	—	—	—	—	—
			1759	5 c.c.	11	26·3·27	Negative	—	—	—	—	—
			2119	5 c.c.	11	26.3.27	*Positive*	Death	—	—	—	—
		3041	2102	5 c.c.	2	12.4.27	Negative	—	—	—	—	—
			2104	5 c.c.	2	12.4.27	Negative	—	—	—	—	—
		2951	1114	2·5 c.c.	1	2.2.27	Negative	—	—	—	—	—
			1671	2·5 c.c.	1	2.2.27	Negative	—	—	—	—	—
			2207	2·5 c.c.	1	2.2.27	Negative	—	—	—	—	—

No.	Method of Inoculation.	Expt. No.	Cattle No.	Quantity of Blood Injected.	Obtained from Donor No.	Date.	Result.		Immunity Test.			
							Transmission of East Coast Fever.	Fate of Animal.	Expt. No.	Date.	Ticks used.	Result.
D	Intraperitoneal...	3057	2122	100 c.c.	3, 12, 13	5.5.27	*Positive*	Death	—	—	—	—
			2193	100 c.c.	3, 12, 13	5.5.27	*Positive*	Recovery	—	—	—	—
				100 c.c.	2122	8.6.27	Negative	—	—	—	—	—
				100 c.c.	7	7.7.27	Negative	—	3199	3.9.27	*Rh. app.* adults (873) and nymphae (899)	Negative.
										5.12.27	*Rh. app.* adults (874)	Negative.
										15.12.27	*Rh. app.* adults (873)	Negative.
										23.12.27	*Rh. app.* adults (901)	Negative.
										6.1.28	*Rh. app.* nymphae (899, 900)	Negative.
										2.2.28	*Rh. app.* nymphae (945)	Negative.
										19.4.28	*Rh. app.* nymphae (911)	Negative.
										10.5.28	*Rh. app.* adults (953) nymphae (1002)	Negative.
			2102	100 c.c.	7	7.7.27	Negative	—	—	—	—	—
			2104	100 c.c.	7	7.7.27	Negative	—	—	—	—	—
E	Subcutaneous and Intradermal	2918	2038	250 c.c. + 2·5	1	20.1.27	*Positive*	Death	—	—	—	—
			1944	250 c.c. + 2·5	1	23.1.27	Negative	—	2966	10.2.27	*Rh. app.* nymphae (847)	Negative.
									3008	18.3.27	*Rh. app.* nymphae (856)	*Positive.*
			2096	250 c.c. + 2·5	1	27.1.27	Negative	—	2966	10·2·27	*Rh. app.* nymphae (847)	Negative.
									3008	18.3.27	*Rh. app.* nymphae (856)	Negative.
										14.4.27	*Rh. app.* adults (857)	*Positive.*
			1199	250 c.c. + 2·5	1	30.1.27	*Positive*	Recovery	—	—	—	—
		2990	1773	250 c.c. + 2·5	9	24.2.27	*Positive*	Death	—	—	—	—
			1894	250 c.c. + 2·5	9	24.2.27	*Positive*	Recovery	2990	30.4.27	*Rh. app.* adults (857)	Negative.
			2054	250 c.c. + 2·5	9	24.2.27	*Positive*	Recovery	2990	30.4.27	*Rh. app.* adults (857)	Negative.
			2076	250 c.c. + 2·5	9	24.2.27	*Positive*	Recovery	3032	4.4.27	*Rh. app.* nymphae (856)	Negative.
										25.4.27	*Rh. app.* nymphae (847, 856) adults (857)	Negative.
										23.5.27	*Rh. app.* adults (857)	Negative.
										14.7.27	*Rh. app.* nymphae (868)	Negative.
										21.7.27	*Rh. app.* nymphae (868)	Negative.
										26.7.27	*Rh. app.* nymphae (869)	Negative.
										3.8.27	*Rh. app.* nymphae (889)	Negative.
										31.8.27	Subc., intrad., and intraperit. injection of E.C.F. blood	Negative.
										28.9.27	Intraj. injection of spleen and gland pulp	Negative.
		3041	2230	100 c.c. + 2·5	2	12.4.27	Negative	—	—	—	—	—
			2192	250 c.c. + 2·5	2	12.4.27	*Positive*	Killed	—	—	—	—

No.	Method of Inoculation.	Expt. No.	Cattle No.	Quantity of Blood Injected.	Obtained from Donor No.	Date.	Result.		Immunity Test.			
							Transmission of East Coast Fever.	Fate of Animal.	Expt. No.	Date.	Ticks used.	Result.
F	Subcutaneous, Intradermal, and into Glands	2920	1776	250 + 2 + 10 c.c.	1	20.1.27	Negative	—	—	—	—	—
			2054	250 + 2 + 10 c.c.	1	23.1.27	Negative	—	—	—	—	—
			1894	250 + 2 + 10 c.c.	1	27.1.27	Negative	—	—	—	—	—
			1196	250 + 2 + 10 c.c.	1	30.1.27	Negative	—	—	—	—	—
G	Subcutaneous, Intradermal, Intraperitoneal, and into Glands	2919	2076	60 + 5 + 60 + 40 c.c.	1	20.1.27	Negative	—	—	—	—	—
			1803	60 + 5 + 60 + 40 c.c.	1	23.1.27	Negative	—	—	—	—	—
			2066	60 + 5 + 60 + 40 c.c.	1	27·1.27	Negative	—	—	—	—	—
			1656	60 + 5 + 60 + 40 c.c.	1	30.1.27	*Positive*	Death	—	—	—	—
H	Subcutaneous and Scarification	3185	2102	25 c.c.	8	13·8.27	Negative	—	—	—	—	—
			2104	25 c.c.	8	13.8.27	Negative	—	—	—	—	—
I	Scarification (with spleen and gland)	3064	1477	—	12	7.5.27	Negative	—	—	—	—	—
					14	10.6.27	Negative	—	—	—	—	—
			1725	—	12	7.5.27	Negative	—	—	—	—	—
					14	8.6.27–9.6.27	Negative	—	—	—	—	—
			1759	—	12	7.5.27	Negative	—	—	—	—	—
					14	8.6.27–9.6.27	Negative	—	—	—	—	—

CONCLUSIONS.

1. In the South African experiments six cattle were injected with virulent blood *into the jugular vein*; all six remained negative. In the North African experiments two cattle were treated in this way and both remained negative.

2. Injection of blood *under the skin*. South African experiments: seven cattle, of which one was positive (with recovery) and six negative. North African experiment: one negative case.

3. Injection *into the skin*. South African experiments: nine cattle, of which one was positive (with death) and eight were negative. North African experiment: one negative case.

4. Injection *into the peritoneal cavity*. South African experiments: four cattle, of which one was definitely positive, another most likely positive, and two negative. North African experiment: one negative case.

5. Injection *into the glands*. This experiment was not carried out in South Africa. In North Africa one animal was treated with negative result.

6. Injection *under the skin* and *into the skin*. South African experiments: 10 cattle, of which seven were positive (two deaths, four recoveries, and one which was killed), two definitely negative, and one (2230) probably also negative. North African experiments: two cattle, of which one was positive (recovery) and one negative.

7. Injection *into the peritoneal cavity* and *into the skin*. This experiment was not carried out in South Africa. In North Africa one animal was done with negative result.

8. Injection *under the skin*, *into the skin*, and *into the glands*. South African experiments: four cattle; all four negative. North African experiments: one positive case.

9. Injection *under the skin*, *into the skin*, *into the peritoneal cavity*, and *into the glands*. South African experiments: four cattle, of which one case was positive (death) and three negative. North African experiments: two cattle, one positive (recovery), one negative.

10. Injection *under the skin* and *rubbing blood into the scarified skin*. South African experiments: two cases, both negative.

11. *Rubbing infected spleen and gland pulp (and blood) into the scarified skin*. South African experiments: three cattle, all negative.

Altogether 49 cattle were used in the South African experiments described above. Of these, 12 gave positive reactions with five deaths and six recoveries (one animal was killed); 36 were negative and one doubtful (probably also negative). Hence we had about 24 per cent. positive cases.

The method of inoculation which gave the best results in our experiments was the injection of blood under the skin and into the skin; with this method we had 70 per cent. successes. Possibly this favourable result must be ascribed to the blood donor (1656) with whose blood nothing but positive results were obtained, even when a simple subcutaneous injection was given. It would seem, therefore, that it is the donor which determines the result of the inoculation. In the case of Donor No. 1656 it is of interest to note that this animal was itself infected by means of blood inoculation.

In the North African experiments 12 cattle were injected with positive results in three cases (all of which recovered) and negative results in nine cases. The percentage of successes was therefore 25. In these experiments the disease seems to have taken a milder course than in our experiments, where nearly 50 per cent. of the positive cases died.

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Dipping as a Method of Eradicating East Coast Fever.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services, and

P. R. VILJOEN, M.R.C.V.S., Dr.Med.Vet., Deputy-Director of Veterinary Services.

I. INTRODUCTION.

Dipping as a method of controlling and eradicating East Coast fever has been practised in the Union of South Africa for more than 20 years, with varying success. For years it has been felt that more accurate information was needed on various aspects of this subject, and hence, when an opportunity presented itself a few years ago of studying the problem under very favourable conditions in the field, a series of experiments was started in the hope of clearing up some of these difficulties. The results of these experiments will be recorded in the following pages.

Dipping of cattle, as a preventive or an eradicative measure against East Coast fever, was started soon after the first appearance of the disease in South Africa. In the Cape a farmer named Douglas started dipping his stock about the year 1898, while, in Natal, Joseph Baynes built a dipping tank in 1901. Baynes persisted in his efforts to free cattle of ticks by dipping, and certainly had considerable success in so far as the eradication of the common blue tick (*Boophilus decoloratus*) was concerned.

At that time dipping at intervals of 14 days was resorted to, and it was generally believed that it was neither safe nor practical to dip cattle at shorter intervals. In 1905 the Principal Veterinary Officer, Natal, referred officially to dipping or spraying as a method of dealing with East Coast fever, and considered it of value only as a preventive before the disease had actually broken out on the farm. Sinclair in an official communication to the Principal Veterinary Officer, Natal, in November, 1906, mentioned that dipping (14 days' interval) had been tried against East Coast fever in Rhodesia, but that it had not checked the disease. Even in the early days of East Coast fever eradication, most persons connected with the work realized that, with an interval as long as 10 to 14 days, dipping could not be relied upon to destroy all the ticks on infected animals, since the larval and nymphal stages of the brown tick remained on their hosts for a period of about three days only. This was one of the main reasons why dipping was not adopted as an official method of dealing with East Coast fever much earlier than was actually the case.

In spite of this disadvantage, we find that the Minister of Agriculture (Natal) issued an Order as long ago as May, 1908, to the effect that all cattle in the *Richmond* infected area had to be *dipped at fortnightly intervals.* During 1909 the Cape Government officially sanctioned the construction of a number of dipping tanks in native areas near the Natal border.

In June, 1908, Pitchford commenced his work on the dipping of cattle; he tested out certain dipping fluids and proved that by using certain strengths of arsenite of soda a dipping interval of five days could be maintained with safety to the animals; at the same time the dip was efficacious in destroying the ticks. Pitchford's dip was stronger than that employed by Baynes, and consequently it was not found necessary to apply special treatment to inaccessible parts (ears, etc.) as was done at Nelsrust (Baynes). Practical experience with Pitchford's 5-day-interval dipping showed subsequently that there was a continuation of infection on East Coast fever farms, in spite of this short-interval dipping; in other words, some infected ticks escaped destruction.

Pitchford then sought to reduce the dipping interval still further, and as a result of further experiments proved that dipping at 3-day (72 hours) intervals could be adopted with safety by reducing the arsenical strength of the dip; such a fluid was still effective in destroying larval and nymphal as well as most adult ticks. He found, further, that with this reduced strength of dip, special treatment of the ears of the animals was necessary, especially to destroy the nymphal ticks which found protection in the waxy substance commonly present inside the ears. Pitchford also proved that this system of dipping or spraying (3-day intervals with hand-dressing of the ears, etc.) was completely effective in preventing infected ticks from dropping off sick animals alive, and thus infecting the farm further. On the other hand, he did not prove experimentally that, by adopting this short-interval dipping on a farm, infected ticks could be kept away from, or rendered harmless on, the dipped animals. This point must be emphasized, since the impression seems general that no East Coast fever infection can be transmitted to animals which are submitted to regular dipping and hand-dressing. In this connection Pitchford himself says: "The farmer will check with certainty, and with little or no loss, an invasion of East Coast fever."

Since the results of Pitchford's classical experiments were published in 1910, dipping with hand-dressing has become the officially recognized method of dealing with East Coast fever in the Union. At first the 3-day interval was adhered to, but later it was found that practically the same results could be obtained with dipping at 5-day intervals.

Generally speaking, dipping as a method of controlling East Coast fever has been quite successful, but there are many defects and practical difficulties connected with the system; these will be discussed at a later stage.

II. DIPPING EXPERIMENTS.

In spite of the possibility of short-interval dipping and the fact that it has been in official use for nearly 20 years, East Coast fever infection is still present in some parts of the Union. If we take as an example the Richmond district of Natal, where dipping has been practised for over 20 years, we find that the disease is still present there to-day on a fairly large scale, and, in a recent outbreak, has caused a very heavy mortality on certain farms.

During the past few years, many instances have come to our notice where, in spite of regular short-interval dipping and hand-dressing, the mortality continued unabated and it was not possible to eradicate all infection on the farm within the prescribed period, of about 15 months; in other words, the dipping did not seem to check the disease, and further infection of ticks apparently took place. As a result of this experience, farmers have become alarmed and have even suggested that the arsenical dips no longer had the same destructive effects on the ticks.

In order to learn more about the effects of dipping on the ticks and on the course of East Coast fever on an infected farm, a series of experiments was planned. These experiments were carried out under natural conditions on an infected farm—and not under laboratory conditions, as was the case with all previous experiments of a similar nature.

The dipping experiments were conducted under the personal direction of the authors, while the detailed study of the effects of the different dips on the ticks themselves was entrusted to Mr. G. A. H. Bedford, Entomologist to the Division. The latter will, therefore, submit a separate report on his observations, and to this frequent reference will have to be made.

(A) CONDITIONS UNDER WHICH THE EXPERIMENTS WERE CARRIED OUT.

For the purpose of these experiments we were able to select a farm, "Kindergoed," district Belfast, Transvaal, which could be considered *ideal* from all points of view.

It was situated in a deep low-lying, warm, moist valley (Elandslaagte valley), on the Pretoria-Komatipoort railway line, and was well fenced.

The farm was surrounded on three sides by high mountains, the summits of which continued on to the high veld; there was, therefore, very little danger of infection spreading in these directions. On the fourth side there was a continuation of the low-lying valley, but the position here was safeguarded by citrus farms well fenced and divided into many fenced camps.

The farm was bisected by the railway line and the Elands River, which ran parallel with the railway; the experimental cattle were kept entirely on the south side of the railway. Apart from this river, the farm was also crossed by a stream which, in the rainy season, was fairly strong and which had to be crossed by the cattle when they were taken to the dipping tanks. This stream may be said to have interfered somewhat with the dipping experiments, but to avoid dipping fluid being washed off the animals' feet when crossing the stream, the cattle were kept away from the water for some hours after dipping.

In order to study the influence of this running water on the dipped cattle, some animals in certain experiments were made to pass through an arsenical footbath every second or third morning before proceeding to the veld.

All cattle were carefully herded while out grazing during the day, and at night were kept in well-constructed kraals. Near the kraals a small fenced camp, a few acres in extent, was constructed, and in this enclosure all sick cattle were kept under close supervision.

When the experiments were started (September, 1927), there was one cattle dipping tank on the farm, but before the end of October a second one had been completed. The low-lying, warm, and moist valley in which the farm is situated is very favourable to tick life and at different times during the course of the experiments ticks were found in abundance. The climatic conditions in the valley were ideal for the propagation of ticks and the spread of East Coast fever.

In September, 1927, the farm was grossly infected with East Coast fever, although at that time the mortality in the local cattle had decreased considerably. The disease was first diagnosed officially in May, 1927, and during the ensuing 30 days no fewer than 30 deaths occurred in a herd of 240 head of cattle, thus indicating that the infection had been present on the farm for some considerable time prior to its discovery. The dipping tank on the farm was out of order and it was not until the 20th July that regular short-interval dipping could be carried out. Two months later (21st September), when the experiments started, only 52 head of cattle were left on the farm, and, as subsequent events proved, most of these had recovered from attacks of East Coast fever. The farm could, therefore, be described as grossly infected.

(B) FIRST SERIES OF EXPERIMENTS.

The first experiment of the series was commenced on the 21.9.27, and the others were started subsequently when further animals had been received; all these experiments were discontinued on the 31.10.27.

Main Object.

The main object of this series of experiments was to determine the preventive action which the official 5-day dipping and hand-dressing had against East Coast fever in cattle exposed on a badly infected farm.

EXPERIMENT No. 1. (S. 3249.)

Duration, from 21.9.27 to 27.10.27.*

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* Although the experiment terminated on this latter date, the cattle were kept under observation for a further period, and many of them which contracted East Coast fever in the course of the experiment died a considerable time after the conclusion of the experiment.

Treatment.—Five-day-interval dipping † and hand-dressing ‡ with tobacco and oil mixture (1 : 10).

Results.—The results of the experiment can conveniently be summarized in tabular form.

TABLE 1.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
320	Pretoria...	21.9.27	All cattle were dipped on day of dispatch from Onderstepoort. Prior to this they had not been dipped for months.	27.9.27	Owing to bad weather conditions, dipping omitted between 21st and 28th days and 33rd and 40th days.	13 days.	Died, E.C.F.
385	,,	,,		,,		18 ,, .	,,
735	,,	,,		,,		14 ,, .	,,
1063	,,	,,		,,		18 ,, .	,,
1161	,,	,,		,,		18 ,, .	,,
1486	,,	,,		,,		—	Negative.
1612	,,	,,		,,		14 days.	Died, E.C.F.
2462	,,	,,		,,		14 ,, .	,,
2478	,,	,,		,,		18 ,, .	,,
2490	,,	,,		,,		19 ,, .	,,
2496	,,	,,		,,		20 ,, .	,,
4145	,,	,,		,,		14 ,, .	,,
4353	,,	,,		,,		15 ,, .	,,

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† By the term "*five-day interval*" is meant a clear period of four days between consecutive dipping days. Thus dipping would be carried out on the 1st, 6th, 11th, 16th, etc., of the month. Similarly "*three-day interval dipping*" means dipping on the 1st, 4th, 7th, 10th, etc., of the month. "*Five-day dipping*" always implies dipping in "*five-day strength*" of a plain aqueous solution of arsenite of soda, i.e. 2 lb. commercial arsenite of soda (containing 80 per cent. As_2O_3) in every 100 gallons of water, or 0.16 per cent. As_2O_3.

"*Three-day strength*" contains 1 lb. arsenite of soda in 100 gallons of water, or 0.08 per cent. As_2O_3; and "*fourteen-day strength*" 3 lb. arsenite of soda, or 0.24 per cent. As_2O_3.

‡ By *hand-dressing* is meant the application by hand of tick-destroying agents to parts of the animal which are not reached with certainty by the dipping fluid in the ordinary course of dipping. The parts which are usually submitted to this treatment are the insides of the ears, the skin between the caudal folds, and round the anus. These parts are wetted to some extent during the process of dipping, but experience has shown that ticks situated there are not killed with any degree of certainty; this is especially the case with larval and nymphal brown ticks that are commonly situated deep down in the cavity of the ears.

Hand-dressing materials are usually made up of a fatty or oily base, in which is included either arsenite of soda or tobacco extract. Quite commonly a colouring agent is added, the advantage being that animals which have undergone treatment can be identified at a glance. More recently the ordinary watery solution of As_2O_3 (14-day dipping strength) has been introduced to replace the oily or fatty dressing.

For the purpose of applying the oily or fatty dressing a paint-brush with a fairly long handle may be used, but any soft absorbent material tied to the end of a stick also serves the purpose.

For applying the aqueous solution, a piece of sheepskin (merino wool) is commonly employed, but any spongy absorbent material held directly in the hand serves the same purpose.

Remarks.—Twelve out of the thirteen animals contracted and died of East Coast fever. From this result it may be concluded that the dipping and hand-dressing as carried out had very little effect in checking the development of the disease. However, the fact must be borne in mind that at the commencement there was an interval of seven days between dippings and again between the 21st and 28th days and 33rd and 40th days. Such extensions of the dipping interval caused by unfavourable weather conditions are, however, very common in practice.

EXPERIMENT No. 2. (S. 3250.)

Duration of the experiment, from 30.9.27 to 27.10.27.

Treatment.—As in Experiment No. 1, the dipping interval was five days and a hand-dressing mixture of tobacco and oil (1 in 10) was employed.

TABLE 2.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
321	Pretoria . . .	30.9.27	All cattle were dipped on day of dispatch from Onderstepoort.	2.10.27	Owing to bad weather conditions, dipping was omitted between 12th and 19th days and 24th and 31st days.	32 days.	Died, E.C.F.
389	,, . .	,,		,,		31 ,, .	,,
426	,, . .	,,		,,		32 ,, .	,,
496	,, . .	,,		,,		25 ,, .	,,
501	,, . .	,,		,,		35 ,, .	,,
565	,, . .	,,		,,		—	Negative.
613	,, . .	,,		,,		—	,,
636	,, . .	,,		,,		17 days.	Died, E.C.F.
639	,, . .	,,		,,		31 ,, .	,,
658	,, . .	,,		,,		17 ,, .	,,
675	,, . .	,,		,,		—	Died, ruptured diaphragm.
880	,, . .	,,		,,		—	Negative.
1068	,, . .	,,		,,		—	Recovered (E.C.F.).
1147	,, . .	,,		,,		19 days.	Died, E.C.F.
1152	,, . .	,,		,,		19 ,, .	,,
1630	,, . .	,,		,,		19 ,, .	,,
1655	,, . .	,,		,,		13 ,, .	,,
1701	,, . .	,,		,,		17 ,, .	,,
2373	,, . .	,,		,,		—	Died (not E.C.F.)
2407	,, . .	,,		,,		34 days.	,,
2492	,, . .	,,		,,		8 ,, .	Recovered (E.C.F.).
2493	,, . .	,,		,,		31 ,, .	Died, E.C.F.
2494	,, . .	,,		,,		—	Negative.
2528	,, . .	,,		,,		—	Died (exposure).
4406	,, . .	,,		,,		19 days.	Died, E.C.F.
4449	,, . .	,,		,,		19 ,, .	,,

Remarks.—In this case 19 out of 26 animals developed East Coast fever within a month and two of these recovered. The animals were dipped on 29.9.27 at Pretoria, and again on the 2.10.27 at Kindergoed, i.e. at an interval of three days. During the course of the experiment the dipping interval had to be extended to seven days on two occasions.

Here, again, it must be concluded that the treatment had no very marked preventive effect.

EXPERIMENT No. 3. (S. 3251.)

Duration, 27 days, i.e. from 1.10.27 to 27.10.27.

Treatment.—Five-day-interval dipping and hand-dressing with tobacco and oil mixture (1 in 10).

TABLE 3.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
404	Pretoria...	1.10.27	All cattle dipped on day of dispatch from Onderstepoort.	2.10.27	Owing to bad weather conditions, dipping was omitted between 11th and 18th days and 23rd and 30th days.	31 days.	Died, E.C.F.
705	„ ..	„		„		—	Recovered (E.C.F.).
718	„ ..	„		„		30 days.	Died, E.C.F.
867	„ ..	„		„		18 „ .	„
1001	„ ..	„		„		—	Negative.
1073	„ ..	„		„		30 days.	Died, E.C.F.
1148	„ ..	„		„		18 „ .	„
1150	„ ..	„		„		18 „ .	„
1151	„ ..	„		„		—	Negative.
1945	„ ..	„		„		18 days.	Died, E.C.F.
2072	„ ..	„		„		30 „ .	„
2092	„ ..	„		„		—	Negative.
2093	„ ..	„		„		24 days.	Died, E.C.F.
2120	„ ..	„		„		—	Negative.
2138	„ ..	„		„		—	„
2169	„ ..	„		„		—	„
2244	„ ..	„		„		—	„
2297	„ ..	„		„		—	„
2500	„ ..	„		„		—	Died (poverty).
1653	„ ..	„		„		18 days.	Died, E.C.F.
3909	„ ..	„		„		18 „ .	„
4523	„ ..	„		„		16 „ .	„
4548	„ ..	„		„		18 „ .	„

Remarks.—Fourteen out of twenty-three animals developed East Coast fever within the 27 days, and of these only one recovered. At the commencement there was no prolonged interval between dippings, the one at Pretoria on the 30.9.27 being followed up by the next at Kindergoed on the 2.10.27. Owing to unfavourable weather conditions, the dipping interval had to be extended to seven days on two separate occasions.

Judging by the heavy mortality, the treatment did not appear to have had any effect on the development of East Coast fever.

EXPERIMENT No. 4. (S. 3252.)

Duration, 22 days, from 5.10.27 to 27.10.27.

Treatment.—In this case five-day-interval dipping and hand-dressing with *sodium arsenite* were adopted (the strength of the arsenic solution in the hand-dressing material was about that of fourteen-day dipping fluid, i.e. 0.24 per cent. As_2O_3).

Table 4.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
635	Pretoria . . .	5.10.27	All cattle dipped on day of dispatch from Onderstepoort.	7.10.27	Owing to bad weather conditions, dipping omitted between 7th and 14th days and 19th and 26th days.	27 days.	Died, E.C.F.
638	,, . .	,,		,,		27 ,, .	,,
879	,, . .	,,		,,		33 ,, .	,,
1801	,, . .	,,		,,		27 ,, .	,,
1863	,, . .	,,		,,		—	Negative.
2095	,, . .	,,		,,		30 days.	Died, E.C.F.
2105	,, . .	,,		,,		—	Negative.
2168	,, . .	,,		,,		—	,,
2170	,, . .	,,		,,		—	,,
2184	,, . .	,,		,,		—	,,
2195	,, . .	,,		,,		—	,,
2202	,, . .	,,		,,		—	,,
2206	,, . .	,,		,,		—	,,
2209	,, . .	,,		,,		—	,,
2218	,, . .	,,		,,		—	,,
2220	,, . .	,,		,,		19 days.	Died, E.C.F.
2221	,, . .	,,		,,		—	Negative.
2263	,, . .	,,		,,		27 days.	Died, E.C.F.
3318	,, . .	,,		,,		20 ,, .	,,

Remarks.—Eight out of nineteen animals contracted East Coast fever within this short period of three weeks.

All cattle were dipped at Pretoria on 4.10.27 and again at Kindergoed on 7.10.27. During the course of the experiment it was necessary to extend the dipping interval to seven days on two occasions.

Here, again, the preventive effect of the treatment did not appear to be very marked.

EXPERIMENT No. 5. (S. 3253.)

Duration of the experiment, nearly a month, i.e. from 29.9.27 to 27.10.27.

Treatment.—Official five-day dipping with hand-dressing (tobacco and oil).

TABLE 5.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
113	Ermelo....	29.9.27	No dipping prior to dispatch.	2.10.27	Owing to bad weather conditions, dipping omitted between 13th and 20th days and 25th–32nd days.	18 days.	Died, E.C.F.
1064	,, ...	,,		,,		27 ,, .	,,
1132	,, ...	,,		,,		14 ,, .	,,
1862	,, ...	,,		,,		11 ,, .	,,
1865	,, ...	,,		,,		16 ,, .	,,
1882	,, ...	,,		,,		14 ,, .	,,
2171	,, ...	,,		,,		16 ,, .	,,
2178	,, ...	,,		,,		11 ,, .	,,
2190	,, ...	,,		,,		16 ,, .	,,
2191	,, ...	,,		,,		12-40 days	Recovered (E.C.F.).
4448	,, ...	,,		,,		16 days.	Died, E.C.F.

Remarks.—Eleven out of eleven or 100 per cent. animals contracted East Coast fever within a month after exposure.

The first dipping was given three days after their arrival on the farm. In this case the treatment certainly had no preventive effect. There was an extension of the dipping interval from five to seven days on two occasions.

The dipping and hand-dressing apparently had no preventive effect.

EXPERIMENT No. 6. (S. 3255.)

The animals in this experiment were obtained from a non-dipping area. Duration, from 13.10.27 to 31.10.27.

Treatment.—Five-day dipping without hand-dressing.

TABLE 6.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
1781	Vryburg...	13.10.27	No dipping prior to dispatch.	19.10.27	Owing to bad weather conditions, dipping omitted between 11th and 18th days.	—	Negative.
1970	,, ...	,,		,,		21 days.	Died, E.C.F.
1997	,, ...	,,		,,		23 ,, .	,,
1998	,, ...	,,		,,		19 ,, .	,,
2006	,, ...	,,		,,		19 ,, .	,,
2009	,, ...	,,		,,		19 ,, .	,,
2018	,, ...	,,		,,		19 ,, .	,,
2030	,, ...	,,		,,		19 ,, .	,,
2082	,, ...	,,		,,		19 ,, .	,,
2085	,, ...	,,		,,		22 ,, .	,,
2089	,, ...	,,		,,		—	Negative.
2090	,, ...	,,		,,		25 days.	Died, E.C.F.

Remarks.—During this short period no fewer than ten out of the twelve animals developed East Coast fever, so that the preventive effect of the treatment was hardly noticeable. The cattle had, however, been on the farm for six days before dipping was started.

During the course of the experiment the dipping interval had to be extended from five to seven days on one occasion.

EXPERIMENT No. 7. (S. 3257.)

Duration of the experiment, from 25.9.27 to 27.10.27.

Treatment.—The cattle had been bred on the farm and exposed to infection ever since the disease had broken out. They had undergone regular dipping during the preceding 2½ months. In the present experiment five-day dipping with hand-dressing (tobacco and oil) was continued.

TABLE 7.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
2514	Local stock	25.9.27	Regularly dipped during preceding 2½ months.	27.9.27	Owing to bad weather conditions dipping omitted between 29th and 36th days.	—	Negative.
2515	,,	,,		,,		—	,,
2516	,,	,,		,,		40 days.	Recovered.
2517	,,	,,		,,		—	Negative.
2518	,,	,,		,,		—	,,
2519	,,	,,		,,		—	,,
2520	,,	,,		,,		13 days.	Died, E.C.F.
2521	,,	,,		,,		15 ,, .	Recovered.
2522	,,	,,		,,		29 ,, .	Died, E.C.F.
2523	,,	,,		,,		24 ,, .	,,
2524	,,	,,		,,		—	Negative.
2525	,,	,,		,,		16 days.	Died, E.C.F.
2526	,,	,,		,,		20 ,, .	,,
2527	,,	,,		,,		24 ,, .	,,

Remarks.—Eight out of the fourteen cattle contracted East Coast fever, and hence the percentage infection was rather smaller than that in some of the other experiments carried out concurrently. These surviving animals did not contract East Coast fever in subsequent experiments, and hence it was concluded that they had developed immunity as a result of a previous attack of the disease.

EXPERIMENT No. 8. (S. 3258.)

Duration of the experiment, from the 19.10.27 to 31.10.27, i.e. only twelve days.

Treatment.—The animals were received from a non-dipping area, but were submitted to weekly dipping for several weeks prior to dispatch.

In this experiment they were dipped every five days (no hand-dressing).

TABLE 8.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Dispatch to Kindergoed.	Dipping Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
120	Besterspuit.	19.10.27	Dipped weekly and on day of dispatch.	24.10.27	Owing to bad weather conditions, dipping omitted between 5th to 12th days.	—	Recovered.
126	”	”		”		—	Negative.
160	”	”		”		—	”
262	”	”		”		—	”
266	”	”		”		—	”
821	”	”		”		—	”
901	”	”		”		—	”
3082	”	”		”		23 days.	Died, E.C.F.
4844	”	”		”		—	Negative.

Remarks.—Two out of the nine animals developed symptoms of East Coast fever soon after discontinuation of the experiment, the infection having undoubtedly been contracted during the course of the experiment.

The experiment did not last long enough to allow of definite conclusions being drawn from the results.

SUMMARY AND CONCLUSIONS.

(*First Series of Experiments.*)

The eight experiments under consideration lasted for varying periods, from 12 days only to a maximum of 36 days. A total of

127 animals were exposed to infection and submitted to treatment; of these 84, or 66 per cent., contracted East Coast fever. The following table gives a short summary of the position:—

Expt. No.	Treatment.		Duration.	No. of Animals.	Cases of E.C.F.	Percentage of E.C.F.
	Dipping Interval.	Hand-dressing.				
1	5 days..	Tobacco and oil......	36 days.	13	12	92
2	5 ,, ..	,, ,, ...	28 ,, .	26	19	73
3	5 ,, ..	,, ,, ...	27 ,, .	23	14	61
4	5 ,, ..	Sodium arsenite......	22 ,, .	19	8	42
5	5 ,, ..	Tobacco and oil......	28 ,, .	11	11	100
6	5 ,, ..	No hand-dressing.....	18 ,, .	12	10	83
7	5 ,, ..	Tobacco and oil......	28 ,, .	14	8	57
8	5 ,, ..	No hand-dressing.....	12 ,, .	9	2	22

From this it will be seen that the largest number of cases occurred in Experiments Nos. 5 and 1 and the smallest number in No. 8. In both Experiments Nos. 5 and 1 tobacco and oil mixture was used as hand-dressing material, but the same treatment was also applied in the case of Experiments Nos. 2 and 7, in which the percentage infection was smaller. Probably no special significance can be attached to the still smaller percentage of cases in Experiment No. 4, in which arsenite of soda solution was used as a dressing material. The small number of cases in Experiment No. 8 is explained by the short duration of the experiment.

Special attention should be directed to Experiment No. 6, in which no hand-dressing was resorted to and where the percentage of infection in the short period of 18 days was 83.

The average duration of the eight experiments was only 25 days, and the percentage infection 66. The latter is almost as high as would be expected on an infected farm where no dipping is carried out. From this result it may be concluded that official five-day dipping with hand-dressing is not necessarily effective in checking immediately an East Coast fever outbreak on a badly infected farm.

Only one further point need be referred to here, namely, the relatively low percentage of infection in Experiment No. 7. The cattle in this experiment had been exposed to infection for several months, during which they had undergone regular dipping. At first we gained the impression that these cattle showed more resistance to infection than the cattle in other experiments and that the prolonged treatment might be responsible for this. However, it was found subsequently that the surviving cattle had apparently developed an immunity against East Coast fever while still in the possession of the local natives.

(C) SECOND SERIES OF EXPERIMENTS.

Some of these experiments were commenced on 27.10.27, others on subsequent dates, and all were discontinued on 1.3.28. Their duration was, therefore, approximately four months.

MAIN OBJECT.

The main object of this series of experiments was to study the comparative results obtained from five- and three-day-interval dipping; at the same time observations were made on the effects of different hand-dressing materials and on the value of clipping the long hairs from the ears and the brush off the tail.

For the dipping plain arsenite of soda in official five- and three-day strength was used. For hand-dressing with arsenite solution, official fourteen-day strength was employed, while for hand-dressing with tobacco and oil, a mixture was made up consisting of 1 part tobacco (2 per cent. nicotine) in 10 parts of mineral oil.

EXPERIMENT No. 9. (S. 3282.)

This experiment lasted for four months, from 31.10.27 to 1.3.28.

Treatment.—Five-day dipping with tobacco and oil hand-dressing was employed, while the ears and tails were regularly clipped.

TABLE 9.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
613	Pretoria...	30. 9.27	*Vide* Experiment : S. 3250	31.10.27	Owing to bad weather conditions, animals not dipped on 14th and 15th January, 1928.	—	Negative.
821	Bestersput.	19.10.27	S. 3258	,,		76 days.	Died, E.C.F.
1486	Pretoria...	21.9.27	S. 3249	..		—	Negative.
1781	Vryburg...	13.10.27	S. 3255	..		—	,,
2218	Pretoria...	5.10.27	S. 3252	,,		87 days.	Died, E.C.F.
2244	Pretoria...	1.10.27	S. 3251	,,		—	Negative.
2517	Local stock	25.9.27	S. 3257	,,		—	,,
2518	,,	,,	S. 3257	,,		—	Died (not E.C.F.).
2519	,,	,,	,,	,,		—	Negative.
2521	..	,,	,,	,,		Immune (Exp. S. 3257)	,,
2560	,,	25.10.27	S. 3287	10.11.27		90 days.	Died, E.C.F.
2582	..	,,	..	..		77 ..	..
2594	,,	21.11.27	—	25.11.27		—	Negative.
2630	Born of C. 2517	13.1.28	—	16.1.28		18 days.	Died, E.C.F.

Remarks.—Five out of fourteen animals contracted and died from East Coast fever, i.e. nearly 36 per cent. The treatment seemed to have some preventive action, but was certainly not effective in warding off the East Coast fever infection in all cases.

EXPERIMENT No. 10. (S. 3283.)

This experiment also lasted for four months, i.e. from 31.10.27 to 1.3.28.

Treatment.—In this case five-day-interval dipping with hand-dressing with sodium arsenite was employed; the ears and tails were clipped regularly.

Table 10.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
880	Pretoria...	30.9.27	*Vide* Experiment : S. 3250	31.10.27	Owing to bad weather conditions, animals not dipped on 14th and 15th January, 1928.	128 days	Died, E.C F.
2092	„ ...	1.10.27	S. 3251	„		105 „	„
2120	„	„	„	„		107 „	„
2184	„	5.10.27	S. 3252	„		116–121 days	Recovered.
2297	„	1.10.27	S. 3251	„		101 days	Died, E.C.F.
2492	„ ...	30.9.27	S. 3250	„		Immune (Exp. S. 3250)	Died, red water (?).
2494	„ ...	„	„	„		—	Negative.
2499	„ ...	8.10.27	S. 3254	„		102 days	Died, E.C.F.
2500	„ ...	1.10.27	S. 3251	„		—	Died, poverty.
2565	Local stock	25.10.27	S. 3288	10.11.27		—	Negative.
1811	Bestersput	1.12.27	Dipped on day of dispatch	5.12.27		—	Died, anaplasmosis.
2248	„	„	„	„		27 days.	Died, E.C.F.

Remarks.—Seven out of twelve animals, i.e. nearly 58 per cent., contracted East Coast fever, and of these one recovered from the disease. It would, therefore, seem as if hand-dressing with arsenic was less effective than with tobacco and oil mixture.

In any case, this method of treatment was not very effective in warding off East Coast fever infection.

EXPERIMENT No. 11. (S. 3284.)

The duration of this experiment was four months, i.e. from 31.10.27 to 1.3.28.

Treatment.—In this case five-day dipping without hand-dressing was employed, but the ears and tails were clipped regularly.

TABLE 11.

Cattle Nos.	Received at Kindergoed.		Treatment prior to this Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
2169	Pretoria...	1/10/27	*Vide* Experiment: S. 3251	31/10/27	Owing to bad weather, animals not dipped on 14th and 15th Jan., 1928.	—	Negative.
2524	Local stock	25/9/27	S. 3259	,,		—	,,
2575	,,	25/10/27	S. 3289	10/11/27		83 days.	Died, E.C.F.
2068	Bestersput.	16/11/27	Subjected to 5-day dipping before dispatch	20/11/27		22 ,,	,,
2233	,,	,,		,,		62 ,,	,,
2235	,,	,,		,,		43 ,,	,,
2238	,,	,,		,,		28 ,,	,,
2595	Local stock	21/11/27	—	25/11/27		41 ,,	,,
2596	,,	,,	—	,,		28 ,,	,,
1949	Bestersput.	1/12/27	Dipped day of dispatch	5/12/27		16 ,,	,,
2254	,,	,,	,,	,,		31 ,,	,,

Remarks.—Nine out of eleven, or nearly 82 per cent., animals contracted and died from East Coast fever.

It is clear, therefore, that five-day dipping alone (without hand-dressing of the ears and tails) has very little effect in protecting animals from East Coast fever when exposed on a badly infected farm.

EXPERIMENT No. 12. (S. 3285.)

This experiment also lasted four months, i.e. from 31.10.27 to 1.3.28.

Treatment.—Five-day dipping and hand-dressing with tobacco and oil mixture; the ears and tails were not clipped.

TABLE 12.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
705	Pretoria...	1/10/27	*Vide* Experiment: S. 3251	31/10/27	Owing to bad weather, animals not dipped on 14th and 15th Jan., 1928.	Immune (Exp. S. 3251)	Negative.
1001	,, ...	,,	S. 3251	,,		—	,,
1068	,, ...	30/9/27	S. 3250	,,		Immune (Exp. S. 3250)	,,
2138	,, ...	1/10/27	S. 3251	,,		105 days	Died, E.C.F.
2168	,, ...	5/10/27	S. 3252	,,		116 ,,	,,
2221	,, ...	,,	,,	,,		—	Negative.
2515	Local stock	25/9/27	S. 3257	,,		—	,,
2569	,,	25/10/27	—	,,		99 days	Died, E.C.F.
2586	Born of C. 1147 (Ex. S. 3250)	12/10/27	—	,,		—	Died (not E.C.F.)
2571	Local stock	25/10/27	S. 3291	10/11/27		—	Negative.
2597	,,	21/11/27	—	25/11/27		47 days	Died, E.C.F.
1864	Bestersput	1/12/27	Dipped day of dispatch	5/12/27		26 ,,	,,
2458	,,	,,	,,	,,		37 ,,	,,

Remarks.—A mortality of six out of thirteen animals, or 46 per cent., occurred, showing that the treatment apparently had some preventive effect. It would seem that leaving the long hairs on the ears and tails undisturbed did not affect the result to any extent. It should be stated that two out of the thirteen animals were immune, having recovered from East Coast fever in a previous experiment.

EXPERIMENT No. 13. (S. 3286.)

This experiment was commenced on 10.11.27 and concluded on 1.3.28, thus lasting over three and a half months.

Treatment.—Five-day-interval dipping combined with hand-dressing with arsenic solution. In this case the ears and tails were not dipped.

TABLE 13.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
613	Pretoria...	30/9/27	*Vide* Experiment: S. 3282	10/11/27	Owing to bad weather, animals not dipped on 14th and 15th Jan., 1928.	62 days.	Died, E.C.F.
1001	,, ...	1/10/27	S. 3285	,,		—	Negative.
2558	Local stock	25/10/27	S. 3287	,,		72 days.	Died, E.C.F.
2559	,,	,,	S. 3289	,,		50 ,,	,,
2570	,,	,,	S. 3287	,,		83 ,,	,,
2587	Born of C. 1001	,,		15/11/27		67 ,,	,,
2118	Besterspuit	16/11/27	5-day dipping prior to dispatch	16/11/27		54 ,,	,,
2124	,,	,,		,,		48 ,,	,,
2255	,,	,,		,,		Recovered (E.C.F.) ?	Died. (R.W. ?).
2242	,,	30/11/27		30/11/27		47 ,,	Died, E.C.F.

Remarks.—A heavy mortality took place, eight out of ten animals, or a percentage of 80, dying from East Coast fever. When comparing these results with those obtained in Experiment No. 12, it would seem as if hand-dressing with arsenic was less effective than with tobacco and oil mixture.

In any case, it may be concluded that this method of treatment is not effective against East Coast fever.

EXPERIMENT No. 14. (S. 3287.)

This experiment was commenced on 27.10.27 and concluded on 1.3.28, thus lasting over four months.

Treatment.—Three-day dipping and hand-dressing with tobacco and oil; at the same time clipping of ears and tails was carried out. Compare Experiment No. 9.

TABLE 14.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
2105	Pretoria...	5/10/27	*Vide* Experiment : S. 3252	27/10/27	Owing to bad weather, animals not dipped on 14th and 15th January, 1928.	—	Negative.
2191	Ermelo....	29/9/27	S. 3252	,,		Immune (Exp. S. 3253)	Died, anaplasmosis.
2195	Pretoria...	5/10/27	S. 3252	,,		118 days	Died, E.C.F.
2514	Local stock	25/9/27	S. 3257	,,		—	Negative.
2558	,,	25/10/27		,,		—	,,
2560	,,	,,		,,		—	,,
2570	,,	,,		,,		—	,,
2573	,,	,,		,,		98 days	Died, E.C.F.
2577	,,	,,		,,		97 ,,	Recovered (E.C.F.).
2582	,,	,,		,,		—	Negative.
1486	Pretoria...	21/9/27	S. 3282	10/11/27		—	,,
2244	,, ...	1/10/27	,,	,,		105 days	Died, E.C.F.
2301	Bestersput	30/11/27	5-day dipping prior to dispatch.	30/11/27		56 ,,	Recovered.

Remarks.—The results are very similar to those obtained in Experiment No. 9, the percentage infection being 36 in the latter compared with 38 in the former.

It would, therefore, seem that there is very little difference in the action of three- and five-day-interval dipping.

EXPERIMENT No. 15. (S. 3288.)

This experiment lasted over four months, from 27.10.27 to 1.3.28.

Treatment.—Three-day dipping and hand-dressing with arsenite of soda and clipping of ears and tails. Compare Experiment No. 10.

TABLE 15.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
266	Bestersput	19/10/27	*Vide* Experiment : S. 3258	27/10/27	Owing to bad weather, animals not dipped on 14th and 15th January, 1928.	—	Died, enteritis.
2516	Local stock	25/9/27	S. 3257	,,		40 days.	Died, E.C.F.
2565	,,	25/10/27		,,		—	Negative.
4844	Bestersput	19/10/27	S. 3258	,,		74 days.	Died, E.C.F.
2592	Local stock	25/10/27		,,		—	Killed, poverty
2494	Pretoria...	30/9/27	S. 3283	9/11/27		93 days.	Recovered.
2259	Bestersput	16/11/27	5-day dipping prior to dispatch.	18/11/27		—	Died (?).
2298	,,	,,		,,		76 days.	Died, E.C.F.
2598	Local stock	21/11/27		21/11/27		57 ,,	,,
2599	,,	,,		,,		49 ,,	,,

Remarks.—Six out of ten animals, or 60 per cent., contracted East Coast fever, and of these one recovered from the disease. The results are, therefore, practically the same as those obtained in Experiment No. 10, thus again showing that there is very little difference in the effects of three-day- as compared with five-day-interval dipping.

EXPERIMENT No. 16. (S. 3289.)

The duration of this experiment was also over four months, from 27.10.27 to 1.3.28.

Treatment.—Three-day dipping alone (without hand-dressing). The ears and tails were regularly clipped. Compare Experiment No. 11.

TABLE 16.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
2202	Pretoria...	5/10/27	*Vide* Experiment S. 3252	27/10/27	Owing to bad weather, animals not dipped on 14th and 15th January, 1928.	116 days	Recovered.
2206	,, ...	,,	,,	,,		40 ,,	,,
2209	,, ...	,,	,,	,,		132 ,,	Died, E.C.F.
2554	Local stock	25/10/27		,,		96 ,,	,,
2559	,,	,,		,,		—	Negative.
2575	,,	,,		,,		—	,,
2576	,,	,,		,,		16 days	Died, E.C.F.
2579	,,	,,		,,		85 ,,	,,
2169	Pretoria...	1/10/27	S. 3284	9/11/27		—	Negative.
1207	Bestersput	30/11/27	Dipped weekly and morning of dispatch.	30/11/27		51 days	Died, E.C.F.
2219	,,	,,		,,		54 ,,	,,
2465	,,	,,		,,		27 ,,	Recovered.

Remarks.—Nine out of twelve animals, or 75 per cent., contracted East Coast fever, and of these three recovered from the disease. The results were, therefore, very much the same as those obtained from five-day dipping, and show that short-interval dipping, without hand-dressing, is not effective against East Coast fever.

EXPERIMENT No. 17. (S. 3290.)

This experiment lasted over four months, from 27.10.27 to 1.3.28.

Treatment.—Three-day dipping alone (without any other treatment).

TABLE 17.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
126	Bestersput	19/10/27	*Vide* Experiment : S. 3258	27/10/27	Owing to bad weather, animals not dipped on 14th and 15th January, 1928.	59 days.	Died, E.C.F.
160	,,	,,	,,	,,		—	Died, poverty.
262	,,	,,	,,	,,		106 days	Died, E.C.F.
565	Pretoria...	30/9/27	S. 3250	,,		—	Negative.
2553	Local stock	25/10/27	—	,,		37 days.	Died, E.C.F.
2557	,,	,,	—	,,		—	Negative.
2562	,,	,,	—	,,		62 days.	Died, E.C.F.
2600	,,	21/11/27	—	21/11/27		26 ,,	,,
2232	Bestersput	31/11/27	5-day dip and on morning of dispatch.	30/11/27		33 ,,	,,

Remarks.—Six out of nine, or nearly 67 per cent., of animals died from East Coast fever. One other animal died from other causes, so that the percentage mortality might have been even higher. The number of animals in the experiment was rather small, but in this case the indications were that three-day dipping without hand-dressing was not less effective than dipping plus hand-dressing.

EXPERIMENT No. 18. (S. 3291.)

This experiment was started on 27.10.27 and lasted until 1.3.28, i.e. over four months.

Treatment.—Three-day dipping combined with hand-dressing with tobacco and oil, but no clipping of ears and tails. Compare Experiment No. 12.

TABLE 18.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
1151	Pretoria...	1/10/27	*Vide* Experiment : S. 3251	27/10/27	Owing to bad weather, animals not dipped on 14th and 15th January, 1928.	112 days	Died. E.C.F.
1863	,,	5/10/27	S. 3252	,,		116 ,,	Recovered.
2089	Vryburg...	13/10/27	S. 3255	,,		114 ,,	,,
2571	Local stock	25/10/27	—	,,		—	Negative.
2581	,,	,,	—	,,		99 days	Died, E.C.F.
2221	Pretoria...	5/10/27	S. 3285	9/11/27		113 ,,	,,
2117	Bestersput	16/11/27	Dipped weekly and on morning of dispatch.	18/11/27		65 ,,	,,
2258	,,	,,		,,		—	Died (?).
1210	,,	30/11/27		30/11/27		9 days	Died. E.C.F.
2174	,,	,,		,,		34 ,,	Recovered.

Remarks.—Eight out of ten animals, or 80 per cent., contracted East Coast fever, and of these three recovered. The percentage infection was, therefore, very high, and it must be concluded that this method of treatment was not effective in warding off East Coast fever. The results were more unsatisfactory than those obtained in Experiment No. 12, but the conclusion would not be justified that the difference was due to the superior qualities of the five-day dipping.

EXPERIMENT No. 19. (S. 3292.)

The duration of this experiment was also over four months, from 27.10.27 to 1.3.28.

Treatment.—Three-day interval dipping combined with hand-dressing with arsenic solution, but no clipping of ears and tails. Compare Experiment No. 13.

TABLE 19.

Cattle Nos.	Received at Kindergoed.		Treatment prior so this Experiment.	Treatment Commenced on.	Dippings Omitted.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.					
120	Besterspuit	19/10/27	*Vide* Experiment : S. 3258	27/10/27	Owing to bad weather, animals not dipped on 14th and 15th January, 1928.	—	Died, poverty.
675	Pretoria...	30/9/27	S. 3250	"		—	Died, ruptured diaphragm.
901	Besterspuit	19/10/27	S. 3258	"		—	Died, poverty.
2170	Pretoria...	5/10/27	S. 3252	"		33 days.	Recovered.
2555	Local stock	25/10/27	—	"		—	Killed.
2556	"	"	—	"		—	Died. snakebite.
2564	"	"	—	"		50 days.	Died, E.C.F.
2568	"	"	—	"		77 "	"
2572	"	"	—	"		—	Died, gastro-enteritis
2223	Besterspuit	16/11/27	Dipped weekly and day dispatched	18/11/27		76 days.	Died, E.C.F.
2601	Local stock	21/11/27	—	21/11/27		26 "	"
2602	"	"	—	"		56 "	"
2196	Besterspuit	30/11/27	Dipped weekly and day of dispatch.	30/11/27		60 "	"
2213	"	"		30/11/27		32 "	"

Remarks.—Eight out of fourteen animals, i.e. 57 per cent., contracted East Coast fever, and of these one recovered. In addition to these, five died from other causes in the course of the experiment. If these deaths had not occurred, it is reasonable to assume that the mortality from East Coast fever would have been still higher.

It must be concluded that this method of treatment is not effective in preventing East Coast fever infection.

SUMMARY AND CONCLUSIONS.

(*Second Series of Experiments.*)

All 11 experiments under consideration lasted for about four months, so that the results can be accepted as fairly definite and as giving a true indication of what may be expected from the different forms of treatment that were applied.

The total number of animals in the 11 experiments was 128, and of these 78, or about 61 per cent., contracted East Coast fever.

There were 60 animals in the five-day, and 68 in the three-day dipping experiments, and the percentage of cases of East Coast fever in these was 60 and 62 respectively. Considering the results as a whole, the conclusion seems justified that in practice there is very little or no difference between five- and three-day-interval dipping.

Regarding the additional methods of treatment, the detailed information supplied in the following summary is instructive:—

Expt. No.	Treatment.		No. of Animals.	Cases of E.C.F.	Percentage of E.C.F.
	Dipping Interval.	Hand-dressing and Clipping.			
9	5 days..	Tobacco and oil + clipping.....	14	5	36
14	3 ,, ..	,, ,, ...	13	5	38
10	5 days..	Arsenite of soda + clipping.....	12	7	58
15	3 ,, ..	,, ,, ...	10	6	60
11	5 ,, ..	Clipping only..................	11	9	82
16	3 ,, ..	,, ...	12	9	75
12	5 ,, ..	Tobacco and oil................	13	6	46
18	3 ,, ..	,, ...	10	8	80
13	5 ,, ..	Arsenite of soda...............	10	9	80
19	3 ,, ..	,, ...	14	8	57
17	3 days..	Dipping only..................	9	6	67

It will be seen that, apart from Experiment No. 17, all the others can be grouped in pairs, and in this way a comparison can be drawn between five-day and three-day-interval dipping in respect of each method.

The following conclusions seem justified in regard to the different additional methods of treatment that were applied:—

(1) Five- or three-day dipping, combined with hand-dressing with tobacco and oil and clipping of ears and tails, appears to have given the best results (Experiments Nos. 9 and 14).

(2) Five- or three-day dipping, combined with hand-dressing with arsenic solution and clipping of ears and tails, is less satisfactory (Experiments No. 10 and 15).

(3) Five- or three-day dipping with clipping, but *without hand-dressing*, is unsatisfactory (Experiments Nos. 11 and 16).

(4) Five- or three-day dipping with hand-dressing with tobacco and oil, but *without clipping*, is also unsatisfactory (Experiments Nos. 12 and 18). Experiment No. 12 appears to be more satisfactory, but on analysing the details of the experiment, it has been found that two of the animals in the experiment were immune and one had died from other causes, leaving only 10 susceptible cattle in the experiment.

(5) Five- or three-day dipping, with hand-dressing with arsenic, is also unsatisfactory (Experiments Nos. 13 and 19). Here, again, the results of Experiment No. 19 appear to be better than those of Experiment No. 13, but on analysing the figures, it is seen that five animals in Experiment No. 19 died from other causes, thus leaving only nine susceptible cattle. It may safely be assumed that, if these five cattle had remained alive, the percentage of East Coast fever infection would have been higher.

(6) In Experiment No. 17 one animal died from other causes, so that the percentage mortality might have been higher had this animal also contracted East Coast fever. It is worthy of note that the percentage infection was not higher in this experiment than in Experiments Nos. 18 and 19, in which hand-dressing, in addition to three-day dipping, was carried out.

(7) During the period of these experiments, 16 control (untreated) animals were exposed to infection on the farm, and of these 15, or about 94 per cent., contracted East Coast fever.

From this it must be concluded that some of the methods of treatment adopted in the experiments had a preventive effect against East Coast fever. This can be said particularly of Experiments Nos. 9 and 14, where hand-dressing with tobacco and oil, combined with clipping of the ears and tails, was carried out; and to a lesser extent of Experiments Nos. 10 and 15, where arsenite solution was used for hand-dressing.

However, this preventive action is not very great, especially when it is remembered that at most only 64 per cent. of the animals (Experiment No. 9) were protected, and this under the ideal conditions obtaining in the experiments. Under these conditions every animal was carefully handled and the treatment applied in the most thorough manner, whereas in practice it is often most difficult, on account of the large number of animals and the lack of assistance, to ensure that the treatment of every animal is carried out properly.

(D) THIRD SERIES OF EXPERIMENTS.

After the three-day dipping in official strength of arsenite of soda solution had been shown, in the previous series of experiments, to have failed in providing absolute protection against East Coast fever infection, it was considered advisable to carry out further tests *with the object of determining whether a more effective dipping fluid could be substituted for the one employed in the previous experiments.*

A test was accordingly arranged in which the effect of the ordinary three-day arsenite of soda dip (0.08 per cent. As_2O_3) could be compared with the effect of the dip originally recommended by Pitchford.

Pitchford's formula reads as follows: To make 400 gallons of dip, use 4 lb. arsenite of soda, 3 lb. soft soap, and 1 gallon paraffin.

EXPERIMENT No. 20. (S. 3450.)

This experiment was started on 1.3.28 and lasted until 21.5.28, i.e. for a period of 2¾ months.

Treatment.—Pitchford's three-day dipping formula (with paraffin and soap) was used, and, in addition, the animals were hand-dressed regularly on every dipping day with tobacco and oil mixture.

TABLE 20.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.				
705	Pretoria...	1/10/27	*Vide* Experiment : S. 3285	1/3/28	—	Killed.
1001	,, ...	,,	,,	,,	—	,,
1068	,, ...	30/9/27	,,	,,	—	,,
1781	Vryburg...	13/10/27	S. 3282	,,	—	Negative.
2515	Local stock	25/9/27	S. 3285	,,	—	,,
2517	,,	,,	S. 3282	,,	—	,,
2519	,,	,,	,,	,,	—	,,
2524	,,	,,	S. 3284	,,	—	,,
2565	,,	25/10/27	S. 3283	,,	—	,,
2571	,,	,,	S. 3285	,,	—	,,
2594	,,	21/11/27	S. 3282	,,	—	,,
102	Klipdrift...	28/2/28	Dipped weekly	,,	28 days.	Died, E.C.F.
605	,,	,,	,,	,,	67 ,,	,,
617	,,	,,	,,	,,	83 ,,	,,
2300	,,	,,	,,	,,	65 ,,	,,
2429	,,	,,	,,	,,	83 ,,	,,
2434	,,	,,	,,	,,	—	Negative.
3961	,,	,,	,,	,,	31 days.	Died, E.C.F.
143	Bestersput.	2/3/28	,,	2/3/28	73 ,,	,,
220	,,	,,	,,	,,	66 ,,	,,
281	,,	,,	,,	,,	—	Negative.
564	,,	,,	,,	,,	—	,,
989	,,	,,	,,	,,	—	,,
990	,,	,,	,,	,,	66 days.	Died, E.C.F.
1112	,,	,,	,,	,,	—	Negative.
3817	,,	,,	,,	,,	32 days.	Died, E.C.F.
4641	,,	,,	,,	,,	77 ,,	,,
1236	Vryburg...	7/4/28	—	7/4/28	—	Negative.
1580	,,	,,	—	,,	34 days.	Died, E.C.F.
1392	,,	,,	—	,,	—	Negative.
1419	,,	,,	—	,,	—	,,
1619	,,	,,	—	,,	—	,,

Remarks.—Of the 32 animals in this experiment, 12 died of East Coast fever, giving a percentage mortality of approximately 37.

During the first part of the experiment very few cases of East Coast fever occurred, since the animals were being grazed on a portion of the farm where apparently infected ticks were not abundant. Towards the end of the experiments cases of East Coast fever became more frequent.

EXPERIMENT No. 21 (S. 3251.)

This experiment was carried out during the same period as the one just described.

Treatment.—In this case plain arsenite of soda (without the addition of soap and paraffin) was used for the 3-day-interval dipping, but hand-dressing with oil and tobacco mixture was carried out exactly as in Experiment No. 20.

TABLE 21.

Cattle Nos.	Received at Kindergoed.		Treatment prior to Experiment.	Treatment Commenced on.	Period between Arrival and First Appearance of Koch's Bodies.	Result.
	From.	On.				
			Vide Experiment :			
565	Pretoria...	30/9/27	S. 3290	1/3/28	—	Killed.
1486	..	21/9/27	S. 3287	"	—	Negative.
2105	"	5/10/27	S. 3287	"	—	"
2169	..	1/10/27	S. 3289	"	—	"
2174	Bestersput	30/11/27	S. 3291	"	—	Killed.
2301	"	"	S. 3287	"	—	"
2465	"	"	S. 3289	"	—	Died, poverty.
2514	Local stock.	25/9/27	S. 3287	..	—	Negative.
2557	"	25/10/27	S. 3290	"	—	"
651	Klipdrift....	28/2/28	Dipped Weekly	"	—	"
719	"	"	"	"	58 days.	Died, E.C.F.
2430	"	"	"	"	76 "	"
2435	"	"	"	"	—	Negative.
4580	"	"	"	"	83 days.	Died, E.C.F.
4717	"	"	"	"	—	Killed, poverty.
4743	"	"	"	"	—	Negative.
1184	Bestersput	2/3/28	"	2/3/28	26 days.	Died, E.C.F.
1192	"	"	"	"	25 "	"
1932	"	"	"	"	—	Killed.
2591	"	"	"	"	80 days.	Died, E.C.F.
4004	"	"	"	"	80 "	"
4591	"	"	"	"	66 "	"
4714	"	"	"	"	80 "	"
4890	"	"	"	"	70 ..	"
977	Vryburg...	7/4/28	—	7/4/28	30 "	Recovered.
1411	"	"	—	"	—	Negative.
1483	"	"	—	"	30 days.	Died, E.C.F.
1566	"	"	—	"	26 "	"
1572	"	"	—	"	—	Negative.

Remarks.—Of the 29 animals in this experiment, 13 contracted East Coast fever, and of these one recovered. The percentage infection was, therefore, nearly 45, or slightly higher than that in the previous experiment.

This difference in the percentage infection is of no significance, especially when compared with the differences observed in the preceding series of experiments.

For all practical purposes it would appear that the addition of soft soap and paraffin to the dip did not increase its protective properties to any marked degree. Both dips failed, in a large percentage of cases, to protect cattle against a natural infection of East Coast fever.

III. GENERAL DISCUSSION.

The results obtained in these experiments are valuable in themselves, but it is proposed to bring under review also the extensive practical experiences with dipping as a method of dealing with East Coast fever which have been gathered in the course of years.

(1) The first question which should be considered is *whether official 5-day dipping combined with hand-dressing is effective in checking an outbreak of East Coast fever on a farm.*

Practical experience in the field has shown that on many occasions cases of East Coast fever continued to occur for several months in spite of regular dipping and hand-dressing. In other cases the treatment appeared to have an almost immediately beneficial effect, inasmuch as the mortality was reduced considerably or ceased altogether.

The experiments recorded above show clearly that *on a badly infected farm* there is no immediate effect on the incidence of the disease, provided that no steps are taken to remove the animals from the known sources of infection. In practice the latter procedure is always adopted, wherever possible, and to this must be ascribed very largely, if not entirely, the sudden cessation of the mortality so often observed. The experiments at Kindergoed were carried out under conditions as good as can be expected on any farm. Every animal was seen daily, and the treatment itself was carried out under the closest supervision.

Rain interfered only on rare occasions, and even then the dipping interval was not longer than seven days.

A running stream on the farm through which the animals had to pass to the dipping tank had to be considered; but, as previously stated, the cattle were kept away from the water for several hours after each dipping. To make up for the dip which might have been washed off the animals' feet while crossing the stream, half the number of cattle in the different experiments were made to walk through an arsenical footbath on every second and third day, i.e. twice during the five days' interval between dippings, while the other half served as controls. The result was that very little difference could be observed in the percentage of infection of the two lots of animals; among those passing through the arsenical footbath, 68 per cent. contracted the disease as compared with 71 per cent. among those not submitted to this treatment.

Such being the case, it is necessary to find an explanation for the fact that the 5-day-interval treatment is apparently not effective in preventing animals on an infected farm becoming infected. The most feasible explanation would seem to be that the interval between two successive treatments is sufficiently long to allow infected ticks to get on to the animals and transmit the infection before the subsequent treatment can be applied. In this connection experiments have shown that larval and nymphal ticks can climb on to animals and engorge in the short period of three days (72 hours) and, in exceptional cases, even in 60 hours. However, during recent years the general belief has been that ticks, getting on to cattle that are regularly dipped, do not bite and suck blood within the first day or two and should, therefore, be caught by 5-day-interval dipping before much harm could be done. This aspect of the question will be considered again.

(2) The next point to consider is *whether 3-day-interval dipping, combined with hand-dressing, is effective in checking an East Coast fever outbreak on an infected farm* by preventing infected ticks from transmitting the disease. We have already seen that 5-day-interval dipping will not succeed, and have to emphasize now that 3-day-interval treatment appears to be in no way superior when judged by the results of our experiments.

There has been a general impression that Pitchford had proved experimentally that, provided cattle were dipped at 3-day intervals, they would not become infected with East Coast fever. However, what Pitchford actually proved was that, by dipping *infected* animals at this interval, ticks could not escape alive and transmit the infection to other cattle, dipped or undipped. In other words, by dipping infected animals regularly on a farm *no further ticks could become infected*. To this point we shall also return later.

Meanwhile, we have to conclude that by carrying out regular 3-day dipping on an infected farm, we cannot hope to succeed in stopping the disease while there are infected ticks and while the animals are exposed to infection. Our observations go to show that ticks readily get on and bite cattle that are dipped at this interval. By the time the next dipping day arrives the ticks have already succeeded in transmitting the infection. Judging by the heavy mortality which occurred in spite of this treatment, we are even forced to the conclusion that, once ticks have got on and attached themselves to the animals, they will succeed in conveying the infection, even if dipping were to take place shortly after. In other words, dipping has no immediate effect on the ticks, which in many cases will engorge fully, transmit the infection while engorging, and then die afterwards.

The results of these experiments bear out in a practical way the findings of Green and Dykman (1918) and of Duke and co-workers (1928), that there is no appreciable accumulation of arsenic in the skin of dipped animals. Had there been such an accumulation, as Pitchford believed, it would hardly be conceivable that the ticks bit so readily and succeeded in transmitting the infection to so large a percentage of the animals.

(3) A further point to consider is, *what dipping fluid is most effective* in preventing cattle from becoming infested with ticks.

It has already been shown that there is very little difference between 3- and 5-day-interval dipping when plain arsenite of soda is used in the official strengths (0.08 per cent. and 0.16 per cent. As_2O_3 respectively). What should be considered now is Pitchford's 3-day laboratory dip, which, in addition to arsenite of soda, contains paraffin and soap. The results of our experiments show that this dipping fluid is no more effective in preventing cattle from becoming infected with East Coast fever than is a dip containing plain arsenite of soda of the same strength.

(4) *Special treatment of the ears and tails of animals, in the form of hand-dressing* with tick-destroying agents, has already received attention, but it is necessary to give further consideration to this very important method of treatment.

It is a well-known fact that the brown tick (*Rhipicephalus appendiculatus*) is the most common and important transmitter of East Coast fever, and it has also been shown that the larval and nymphal brown ticks are to be found in the ears only. It follows, therefore, that, if the disease is to be checked on a farm, special attention should be paid to the destruction of these larval and nymphal ticks. By their destruction the adult infected tick, which is the more difficult to kill, is eliminated.

This fact has received official recognition for some years, and hand-dressing of the ears and tails of cattle on infected farms has been enforced under the East Coast fever regulations. Although hand-dressing has been practised for many years, there have been many cases where, in spite of the regular application of this additional method of treatment, outbreaks of East Coast fever have not been checked for a considerable time.

The results of the experiments under discussion have shown clearly that hand-dressing is of definite value in combating East Coast fever on an infected farm, while Bedford made the observation that either 5- or 3-day dipping alone (without hand-dressing) was not effective in destroying the larval and nymphal ticks in the ears.

Regarding the best material to be used for this purpose, the results of these experiments would seem to indicate that a tobacco and oil mixture was superior to an arsenical solution. In the Union the selection of the hand-dressing material is left to the local Government Veterinary Officer, and this has resulted in a great variety of preparations being used. We were not in a position to test out a large number of hand-dressing materials, but selected for our experiments two of the preparations most commonly employed. In practice great reliance is placed by some officers on different kinds of greasy preparations, the so-called "tick greases," but in one case, which came under our observation, the use of tick grease gave no better results than was obtained from other preparations. In this case the animals were dipped twice weekly (i.e. every fourth and third day) in 5-day-interval dip and were regularly hand-dressed with "tick grease" on each dipping day, but deaths from East Coast fever continued unabated for several months.

While there appears to be no doubt that careful and regular hand-dressing will be effective in destroying most or all larval and nymphal brown ticks, the fact remains that in our experiments, cases of East Coast fever continued to occur, even when this treatment was carried out at 3-day intervals. It is difficult to explain this in any other way than the one we have already ventured to express, namely, that ticks are not destroyed immediately by the treatment, but live sufficiently long to transmit the disease to their hosts even when these are being dipped and hand-dressed at 5- or 3-day intervals.

(5) *Clipping of the long hairs from the ears and brush of the tail* has been in official use for some years, the underlying idea being to make these parts more accessible to the dipping fluid or the hand-dressing material. As far as the tail brush is concerned, it would seem that fewer ticks are to be found on tails that have been clipped than on unclipped tails. The results of the dipping experiments also indicate that tick destruction is more effective when clipping of ears and tails is carried out, but the difference is not very great. In practice this clipping involves a great deal of labour, but it is essential to remove the long hairs at the entrance to the ear, especially when a greasy preparation is used for the treatment of the ears.

(6) Another question to be answered is, *whether regular short-interval dipping on an infected farm is effective in preventing further ticks from becoming infected* with East Coast fever parasites. If this is so, then the disease must disappear within about 15 months, since we know from former experiments, and from the experience of the last 25 years, that the brown ticks will not live or remain infective for more than 14 or 15 months under veld conditions.

And yet there are many cases on record where deaths from East Coast fever continued for 18 months, two years, or even longer, in spite of regular dipping and hand-dressing.

Now we know from Bedford's observations that all larvae and nymphae are destroyed by proper dipping and hand-dressing; and we also know that the infection does not pass through the egg of the tick. If, therefore, cases of East Coast fever continue to occur after 15 months of dipping and hand-dressing, we can only conclude that fresh ticks must have become infected during that period.

Theoretically this should not be possible; in other words, if every animal on an infected farm were subjected to thorough dipping and hand-dressing at regular intervals of three or five days, there should be no further infection of ticks and the disease should be at an end after 15 months. But this ideal is difficult to attain. In practice many difficulties arise which may be described as the inherent imperfections of the dipping system. Some of these may be referred to briefly:

(*a*) When dealing with large herds of cattle it becomes almost a physical impossibility to attend carefully to each animal, on every third or fifth day, for a long continuous period. The result is that the work is carried out imperfectly at times (and this has aptly been ascribed to the failure of the "human element").

(*b*) On wet or rainy days dipping cannot be carried out, and the interval must therefore be lengthened. Unfortunately, rainy or wet days, on which dipping has to be postponed are not uncommon during the summer months.

(*c*) During the winter months, again, cattle are very often in poor condition, with the result that some animals have to be exempted from regular dipping or else the dipping interval has to be increased.

(*d*) On some farms it is a difficult matter to bring all cattle regularly to the dipping tank at stated intervals, with the result that some escape regular immersion; we refer particularly to farms that are unfenced, thickly wooded, or whose owners are not sufficiently careful.

(*e*) It is sometimes very difficult to get sick cattle to the dipping tank; such cattle may be some distance from the tank and can be driven there only with great difficulty. If they happen to be suffering from East Coast fever, it stands to reason that further infection of ticks might take place. There are standing instructions that sick cattle on an infected farm must either be dipped regularly or destroyed, but it is not always possible to carry this out promptly.

These difficulties are very common in practice, and make the dipping method a very imperfect system of eradicating East Coast fever from an area.

(7) A further point that received attention was, *whether the tick-killing effects of dipping were more pronounced in cattle that have been dipped regularly for some time than in animals only recently introduced to dipping.* Our observations have shown that ticks readily attach themselves to cattle that have been dipped regularly over long periods. In the experiments recorded in this article some animals were used which had been dipped for several months, others for a few weeks only, and still others not at all. East Coast fever appeared among all of them, and the incidence of the disease was approximately the same in all the groups. It is true that in the first series of experiments the impression was gained that the mortality was smaller in those cattle which had been dipped for a long period, but it was soon discovered that the smaller percentage of infection in the local cattle was not due to prolonged dipping, but to the fact that a big percentage of them had developed immunity as a result of natural attacks of the disease.

(8) From the results of these experiments, and from observations in the field, it may be concluded that *dipping is by no means an ideal method of dealing with an outbreak of East Coast fever.*

To prevent misunderstanding, we should add at once that we do not question for one moment the value of dipping as a method of tick destruction. Dipping has brought untold benefits to South Africa and other countries where tick life is abundant. In our country dipping has reduced greatly the incidence of diseases like redwater and gallsickness in cattle, biliary fever in horses and dogs, and heartwater in cattle, sheep and goats. It has rendered dairy farming possible and lucrative in areas where formerly it was impossible to raise calves and where it was rare to find a cow with all four teats intact. It has rendered large tracts of country available for sheep-farming where for decades no sheep could be kept owing to the ravages of heartwater. It has also assisted materially in the campaign against East Coast fever, inasmuch as it has reduced the number of ticks enormously and thus made it possible to suppress an outbreak of the disease more easily.

Without dipping cattle, farming would be an impossibility in South Africa to-day.

But as a method of eradicating East Coast fever, it has its serious drawbacks, the chief of which is the slowness of the process. We have seen above that cases of East Coast fever may be expected as long as there are infected ticks on the farm, and even after the last death it is necessary to continue short-interval dipping for at least 15 months. During this long period cattle may stray or be moved illegally from the infected farm, and if one of the animals happens to be infected, fresh outbreaks of the disease (very often unsuspected and undetected for a considerable time) may occur in some other part of the country.

Another drawback of the dipping method is its great cost to the State. In practice it has been found necessary to provide supervision in all cases where compulsory dipping is carried out, and this has meant the maintenance of a very large staff of State-paid supervisors. If satisfactory results were assured within a reasonably short period, the expenditure would be completely justified, but, in view of the uncertainty of the results, the question must be asked whether other methods would not yield better and more certain results in a shorter time and at a lesser cost.

One such method which immediately suggested itself is the *slaughtering of all cattle or their removal* from the infected farm as soon as the infection is diagnosed. Such a method may seem crude and costly, but it is ideal from many points of view, and, in the long run, is far cheaper than dipping under supervision. By slaughtering or removing all cattle and keeping the infected farm free of cattle for a period of at least 15 months, all danger of a further spread of the disease is eliminated, the cleansing of the farm at the end of that period is assured, and the costs and trouble of 15 months' dipping at three or five days' intervals are saved.

Nevertheless, dipping will still be used in the campaign against East Coast fever in many cases. As a preventive method it will continue to serve the useful purpose of reducing the number of ticks; and in actual outbreaks of East Coast fever there may be circumstances which may render other measures impracticable and leave no alternative but to enforce short-interval dipping.

However, in cases where dipping has to be employed for the control of East Coast fever, it is well to be aware of the drawbacks and limitations of this method. The experiments recorded in this paper have shown that we cannot expect dipping to put a stop to the mortality on a farm which has become badly infected with East Coast fever. In such a case, therefore, the best policy, especially if the outbreak is an isolated one, would be, *not* to enforce dipping, but to adopt some other measure (such as slaughtering) which would eliminate the risk of a further spread of the disease.

IV. SUMMARY AND CONCLUSIONS.

(1) Short-interval dipping with hand-dressing has been shown not to be effective in checking an outbreak of East Coast fever on an already badly infected farm. Cases of the disease will occur so long as there are infected ticks on the farm and the cattle are exposed to infection by such ticks.

(2) There appears to be very little difference in the effects of 3- and 5-day-interval dipping and hand-dressing.

(3) The value of dipping in plain arsenite of soda of 3-day strength (0.08 per cent. As_2O_3) is not materially enhanced by adding to this fluid soap and paraffin (Pitchford's formula).

(4) Hand-dressing of the ears and tails appears to be a necessary adjunct to dipping for the destruction of all ticks on animals. Of the preparations tried, a tobacco and oil mixture has given the best results.

(5) The clipping of hairs from the ears and brush of the tail seems to assist dipping and hand-dressing in the destruction of ticks.

(6) Cattle which have been dipped regularly for a considerable time seem to contract East Coast fever as readily as those only recently dipped for the first time. This result speaks against the idea that there is an appreciable accumulation of arsenic in the skin of dipped animals.

(7) On many infected farms where short-interval dipping is carried out, further infection of ticks takes place, this being due mostly to the many practical difficulties in the way of carrying out the system in a perfect manner. Among these difficulties may be mentioned unfavourable climatic conditions, poor grazing, lack of fencing, and proper control of the animals.

(8) In the destruction of ticks and the eradication of tick-borne diseases dipping has been of inestimable value; it has rendered many parts of the Union fit for cattle and sheep farming.

(9) For the eradication of East Coast fever dipping is a slow process which has many disadvantages. With the present very limited distribution of the disease in the Union it would pay the State much better to adopt other measures (e.g. slaughtering or removal of cattle), especially in isolated outbreaks.

Section II.

Virus Diseases.

P. J. Du Toit ... Nature and Duration of Immunity against Bluetongue in Sheep.

P. J. Du Toit ... Studies on the Virus of Bluetongue.

15th Annual Report of the Director of Veterinary Services, Union of South Africa. October, 1929.

The Nature and Duration of the Immunity Against Bluetongue in Sheep.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services, Onderstepoort.

INTRODUCTION.

VERY little has been published during the last twenty years on the subject of bluetongue in sheep. The investigations of Theiler, Spreull, and others, during the early years of this century, cleared up most of the problems in connection with this disease, and enabled veterinary research workers in South Africa to devote their attention to other more pressing problems.

The method of immunization introduced by Theiler in 1906 has proved eminently satisfactory and has been a veritable blessing to sheep farmers in those parts of the country where bluetongue occurs. Since the introduction of this vaccine more than 25 million doses have been issued and the results, generally speaking, have been very good indeed. Unfortunately, it is impossible to obtain reliable data about the results of inoculation carried out over the length and breadth of the country by the farmers themselves; it is, however, probably safe to assume that the total losses from bluetongue in vaccinated sheep amounted to less than one per cent.

Nevertheless, cases have been reported every year of unsatisfactory results following the use of bluetongue vaccine. In some cases the vaccine was supposed to cause unduly severe reactions in inoculated sheep; in others it was supposed to produce insufficient immunity, so that such sheep contracted virulent bluetongue when exposed to natural infection. The first class of complaint need not be discussed here in detail. Suffice it to say, that, whenever such complaints could be investigated, it was found that other causes and not the bluetongue vaccine were responsible for the severe reactions or the mortality in the sheep. Thus in many cases it was found that the sheep had been exposed to and become infected with natural bluetongue before they were vaccinated, and that the mortality was due to the virulent disease. In many other cases the sheep were found to be heavily infested with worm parasites from the effects of which they were actually dying, or were reduced to such a state of weakness that even the mild vaccine reaction affected them adversely.

In regard to the second class of complaint, namely that insufficient protection had been afforded by the vaccine, it was felt that more information was required about the nature of the immunity produced by the vaccine. It was largely the realization of our inadequate knowledge on this point which prompted the investigations detailed in the following pages.

In the past, farmers in bluetongue areas have been advised to inoculate their sheep every year, the idea being that the immunity resulting from the vaccine (or, for that matter, from a natural attack of bluetongue) will have disappeared after this period. In actual practice many farmers found that there was still some degree of immunity present after one year and, therefore, they gave the full dose of vaccine (1 c.c.) to their sheep only at the first inoculation, and half a dose (0.5 c.c.) each subsequent year.

Theiler (1909) carried out a few experiments on this point and found that the immunity could be broken down by comparatively large doses of virus (20 to 100 c.c.) and that the breakdown was the more likely to occur the longer the period between immunization and test. He also observed natural breakdowns 10 to 15 months after immunization.

EXPERIMENTAL WORK.

Our own experiments were intended to give more accurate information on this problem.

Object of the Experiments.

To determine the duration of the immunity conferred on sheep inoculated with the ordinary bluetongue vaccine; and also the degree of the immunity present after varying intervals when tested with the homologous vaccine or with a virulent (heterologous) strain.

Method Employed.

Out of the large number of sheep (nearly 2,000) used during the 1927 season for the production of bluetongue vaccine, about 130, all of which had given very good reactions, were selected and retained in order to be tested for immunity after varying intervals. In some of these tests the same strain of virus (vaccine) was used as in the original inoculation, in other cases a virulent (heterologous) strain was employed.

In all of these experiments the standard dose of 1 c.c. vaccine or virus was given subcutaneously. From Theiler's experiments it was known that large doses of virus could break down the immunity; in our experiments, however, the object was to use the smaller dose which would approximate more closely to the conditions of a natural infection.

EXPERIMENT 1. (S. 3462.)

Immunity Test Four Weeks after Immunization.

Ten immune sheep were used in this experiment. All of these had given a good temperature reaction when injected with the vaccine strain of bluetongue (B. 575 and 576). The temperature reaction lasted, in practically all cases, from the 7th or 8th to the 15th or 16th day after injection. Two weeks after the return of the temperature to normal (i.e. four weeks after the first inoculation) the immunity of these sheep was tested. Six were injected with a fairly virulent strain of bluetongue virus (No. 16357) and four with the same vaccine strain with which they had been injected previously. Four controls were added; two were injected with the virulent strain and two with the vaccine strain.

Result.—None of the ten immune sheep reacted. All four control sheep gave good temperature reactions.

Conclusion.—The immunity conferred by the bluetongue vaccine appears to be solid four weeks after the vaccination.

EXPERIMENT 2. (S. 3495.)

Tested after Two Months.

The arrangement was the same as in the previous experiment. Nine sheep were tested for immunity two months after the first inoculation. Five were injected with the virulent strain, together with two controls; four immune and two control sheep were injected with the vaccine strain (B. 577 and 578).

Result.—Of the five sheep tested with the virulent strain one gave a good temperature reaction but showed no other clinical symptoms of bluetongue. The two control sheep which were tested with the same strain both reacted severely and one of them died.

None of the four sheep injected with the vaccine strain reacted; the two controls gave temperature reactions, but showed no other symptoms.

Conclusion.—In the majority of cases (eight out of nine) the immunity appeared to be solid two months after the first inoculation. In one case, however, a sheep (No. 19511) reacted to the injection of a virulent strain although two months previously it had also given a good reaction when injected with the bluetongue vaccine.

It would appear, therefore, that in a small percentage of cases (one out of nine) the immunity produced by the vaccine may have disappeared partially after two months.

EXPERIMENT 3. (S. 3525.)

Tested after Three Months.

Fifteen sheep which had passed through the immunizing reaction three months previously were used in this experiment. Nine were tested with a virulent strain together with three controls; and six immune and three control sheep were tested with the vaccine strain (B. 579, 580, and 581).

Result.—None of the 15 immune sheep reacted, whereas all six control sheep gave good temperature reactions.

Conclusion.—In all the animals tested in this experiment the immunity conferred by the vaccine seemed to be solid after three months.

EXPERIMENT 4. (S. 3337.)

Tested after Four Months.

Ten sheep which had been inoculated with bluetongue vaccine four months previously and two control sheep were tested with a virulent strain.

Result.—The two controls reacted severely and one of them died of bluetongue. Of the 10 immunized sheep, five showed distinct temperature reactions; in three the temperature reaction was somewhat doubtful and two remained definitely negative.

Conclusion.—In these ten sheep the immunity had partly disappeared four months after immunization. However, it is of importance to note that whereas the two control sheep reacted very severely and one actually died, the reactions in the immunized sheep, if present at all, were confined to a moderate rise of temperature. It would seem, therefore, that the immunity in these sheep, although not strong enough to prevent a temperature reaction, was sufficiently strong to protect the sheep against any serious effects of a virulent bluetongue strain.

EXPERIMENT 5. (S. 3373.)

Tested after Five Months.

Twenty immunized sheep were tested, 10 with a virulent strain and 10 with the same vaccine strains (B. 530, 531, 532, 533, and 534) with which these sheep had been immunized five months previously. Three control sheep were added to the former lot and two to the latter.

Result.—Out of the 10 sheep injected with the virulent strain (16357) five gave typical temperature reactions, without any other symptoms, three gave somewhat doubtful reactions, and two remained negative. The three control sheep which were tested with the same strain showed very severe clinical symptoms of bluetongue and one of them died of bluetongue.

Of the 10 sheep tested with the vaccine strains, four showed good temperature reactions, one a mild reaction, two doubtful reactions, and three remained negative. No clinical symptoms were observed in any of these sheep. The two controls showed good temperature reactions, but no clinical symptoms (as was to be expected with a vaccine strain).

Conclusion.—Taking the 20 sheep together, we see that the immunity, which had been present 4½ months previously, had largely disappeared. Only five sheep (25 per cent.) failed to react at all when tested, five gave doubtful reactions, and the other 10 (50 per cent.) showed distinct bluetongue reactions, comparable with those shown five months ago.

The interesting fact again is that, although these sheep gave temperature reactions, no clinical symptoms of any kind were observed, in spite of the fact that half of them were tested with a virulent strain which killed one out of the three control sheep.

There is, therefore, undoubtedly still a partial immunity present after five months, which is sufficient to protect the sheep, in a clinical sense, against bluetongue.

EXPERIMENT 6. (S. 3414.)

Tested after Six Months.

Twenty-five immunized sheep were used in this experiment, 15 were tested with a virulent strain (16357), together with three controls, and 10 plus two controls were tested with the vaccine strains (B. 543, 544, 545, 546, and 547) with which these 10 sheep had previously been inoculated.

Result.—Of the 15 sheep tested with the virulent strain, seven or eight gave distinct temperature reactions, but none of them showed any clinical symptoms. The remainder were either negative or very doubtful. The control sheep showed fairly severe reactions.

Of the 10 sheep tested with the vaccine strains one gave a typical reaction, one a late reaction, one a mild reaction, two doubtful reactions, and the other five were negative. The two control sheep gave good reactions.

Conclusion.—It would appear that the immunity conferred by the vaccine was still "solid" in about 50 per cent. of these sheep. The other 50 per cent. were again susceptible in varying degrees, but not one of them showed clinical symptoms on being tested with a virulent strain.

EXPERIMENT 7. (S. 3515.)

Tested after Nine Months.

Twenty sheep which had been immunized nine months previously were tested; 10 with a virulent strain of bluetongue (16357) and 10 with the original vaccine strains (B. 556, 557, 558, 560, and 561). Three controls were added to the first lot and two to the second.

Result.—Of the 10 sheep tested with the virulent strain five showed no reaction at all; the other five showed good temperature reactions, in one or two cases (18204, 18222), with slight clinical symptoms of bluetongue (feet slightly warm and tender, lips red, and swollen). Two out of the three controls reacted with clinical symptoms, the third remained negative.

Of the 10 sheep tested with the vaccine strains none reacted. The two controls gave temperature reactions.

Conclusion.—The result of the test with virulent material shows that after nine months the immunity is still further reduced so that slight clinical symptoms may appear in some of the sheep. However, even after this interval 50 per cent. of the sheep so tested were found to be completely immune.

The result of the test with the vaccine strain was somewhat surprising and probably misleading, in that not one of the tested animals reacted. In the foregoing experiments it was found that even after five or six months a certain percentage of sheep so tested must be expected to react. The negative result in this case must probably be explained as a coincidence due to the small number of sheep employed.

EXPERIMENT 8. (S. 3609.)

Tested after Twelve Months.

Again 20 sheep were used; 10 of these were tested with a virulent strain and 10 with the vaccine strain (B. 558, 559, 561, 562). All these sheep had been immunized exactly twelve months previously. Three controls were added to each group.

Result.—Of the 10 sheep tested with the virulent strain four were entirely negative, two gave very slight rather doubtful temperature reactions, and only four showed definite typical temperature reactions, but no other clinical symptoms. The three controls reacted typically and one showed severe clinical symptoms, but recovered.

Of the 10 sheep tested with the vaccine strain four were entirely negative, five gave slight very mild temperature reactions, and only one showed a good temperature reaction. The three controls gave the usual reactions without clinical symptoms.

Conclusion.—It will be seen from the above results that, even a full year after vaccination, a fairly large percentage of sheep are completely immune, not only against the homologous vaccine strain, but even against a virulent strain. Those sheep that did react showed (in the present experiment) only a rise of temperature without any other clinical symptoms.

It would seem, therefore, that, as a rule, the vaccine protects sheep for a period of at least a year against serious illness and death from bluetongue.

SUMMARY.

In the following table the results of the experiments recorded above have been summarized.

Exp. No.	Interval between Immunization and Immunity Test.	Sheep Tested with—	Number of Sheep.		Result.		Percentage of Reactors.	Remarks.	Total No. of Immune Sheep.	Percentage of Reactors Among Immune Sheep.	*Total No. of Control Sheep.*	*Percentage of Reactors Among Control Sheep.*
			Immune Sheep.	*Control Sheep.*	Number of Reactors.	Negative.						
1	1 month.....	Virulent Strain.	6		0	6	0%					
				2	*2*	*0*	*100%*	*1 death.*				
		Vaccine Strain.	4		0	4	0%		10	0%		
				2	*2*	*0*	*100%*				*4*	*100%*
2	2 months....	Virulent Strain.	5		1	4	20%					
				2	*2*	*0*	*100%*	*1 death.*				
		Vaccine Strain.	4		0	4	0%		9	11%		
				2	*2*	*0*	*100%*				*4*	*100%*
3	3 months....	Virulent Strain.	9		0	9	0%					
				3	*3*	*0*	*100%*					
		Vaccine Strain.	6		0	6	0%		15	0%		
				3	*3*	*0*	*100%*				*6*	*100%*
4	4 months....	Virulent Strain.	10		5	5	50%	No clinical symptoms.	10	50%		
				2	*2*	*0*	*100%*	*1 death.*			*2*	*100%*

Exp. No.	Interval between Immunization and Immunity Test.	Sheep Tested with—	Number of Sheep.		Result.		Percentage of Reactors.	Remarks.	Total No. of Immune Sheep.	Percentage of Reactors Among Immune Sheep.	*Total No. of Control Sheep.*	*Percentage of Reactors Among Control Sheep.*
			Immune Sheep.	*Control Sheep.*	Number of Reactors.	Negative.						
5	5 months....	Virulent Strain.	10		5	5	50%	No clinical symptoms.	20	50%	*5*	*100%*
				3	*3*	*0*	*100%*	*1 death.*				
		Vaccine Strain.	10		5	5	50%					
				2	*2*	*0*	*100%*					
6	6 months....	Virulent Strain.	15		8	7	53%	No clinical symptoms.	25	44%	*5*	*100%*
				3	*3*	*0*	*100%*	*Severe reactions.*				
		Vaccine Strain.	10		3	7	30%					
				2	*2*	*0*	*100%*					
7	9 months....	Virulent Strain.	10		5	5	50%	Slight clinical symptoms.	20	25%	*5*	*80%*
				3	*2*	*1*	*66%*	*Severe reactions.*				
		Vaccine Strain.	10		0	10	0%					
				2	*2*	*0*	*100%*					
8	12 months...	Virulent Strain.	10		6	4	60%	No clinical symptom	20	60%	*6*	*100 %*
				3	*3*	*3*	*100%*	*Severe reactions.*				
		Vaccine Strain.	10		6	4	60%					
				3	*3*	*0*	*100%*					

DISCUSSION AND GENERAL CONCLUSIONS.

1. The *immunity* conferred by the bluetongue vaccine seems to be "solid" in practically all cases for a period of *at least three months.*

2. *After four months* the immunity seems to become weaker and some of the immunized sheep may now give a temperature reaction if injected with a virulent strain of bluetongue virus.

3. *Six months* after immunization the immunity seems to be practically the same as after four months. Sheep tested with a virulent strain or with an attenuated strain of bluetongue virus may show temperature reactions in approximately 50 per cent. of the cases, but none of the sheep so tested in our experiments have shown any other clinical symptoms of the disease.

4. Hence, for a period of at least six months, the immunity appears to be strong enough to protect sheep in a clinical sense. If immunized sheep should be exposed to reinfection during this period they may develop a fever reaction, but will not show other symptoms of the disease. *No losses need be feared.*

5. *After nine months* there is still a considerable degree of immunity present. Approximately half of the immunized sheep will still have a solid immunity. However, in the other half, the immunity may now be weaker than after six months, so that sheep which are reinfected at this stage may, in addition to a fever reaction, show *slight clinical symptoms* of bluetongue. In the experiments none of the sheep showed serious symptoms nor were their lives in any way endangered.

6. *Twelve months after immunization* with bluetongue vaccine *the immunity of sheep is still fairly strong.* When injected with virulent bluetongue virus only a certain proportion of the sheep will react and these will generally show a fever reaction only. According to the results of the experiments no losses need be feared in such sheep.

7. Generally speaking, there was a *gradual but slow decrease in immunity* from the third to the twelfth month after vaccination. But even at the latter stage the immunity was strong enough to prevent losses.

8. The marked *difference* which was shown throughout the experiments *between immunized and susceptible (control) sheep* proves beyond doubt the value of the vaccine. Of the 21 susceptible sheep injected with the virulent bluetongue strain, practically every one gave a severe fever reaction, in many cases with clinical symptoms, and four died; whereas of the 75 immunized sheep tested with the same strain, only 20 showed a rise of temperature, and of these not one was at any time in danger of dying.

9. On the whole, there was not much *difference* between the reactions in immunized sheep produced by the *virulent strain* and those produced by the *vaccine strain.* There was, of course, a very marked difference in the reactions produced by these two strains in the *control sheep*; whereas the virulent strain caused severe temperature reactions with clinical symptoms and death, the vaccine strain

produced only the comparatively mild temperature reactions usually experienced after vaccination.

10. From the results of these experiments the conclusion seems justified that *vaccination once a year* is sufficient to protect sheep against bluetongue. It was shown in the experiments that even at the end of the 12 months' period there was sufficient immunity present to render the injection of 1 c.c. virulent bluetongue virus harmless.

11. There would seem to be some justification for the practice adopted by many farmers, and alluded to at the beginning of this article, of giving their sheep only *half a dose* (½ c.c.) of vaccine each year after the first inoculation. However, it would be safer and in every way preferable to continue giving the full dose.

12. Another practice of some farmers may be analysed in the light of the foregoing experiments. In bad bluetongue areas the sheep are sometimes *inoculated twice in one year*; first just before the "bluetongue season" (say in October), and then again just before the heavy rains (say in January). This practice has sometimes been recommended by veterinary officers. It finds its explanation and justification in the above experiments; the immunity conferred by the first inoculation would begin to decline just at the time (after three or four months) of the heavy rains when bluetongue is generally at its worst; hence the second inoculation. However, if sheep are otherwise in good health and condition, it would not seem necessary to give this second inoculation. Our experiments have shown that, even if the sheep should get infected with a virulent strain three or four months after vaccination, their immunity will be strong enough to protect them. It is only in cases where the sheep's vitality and resistance have been lowered through drought or worm infection, or other diseases, that a second inoculation with bluetongue vaccine may be advisable.

13. In interpreting the above results, it is necessary to bear in mind that all the experiments were conducted under "*laboratory conditions*"; the sheep were stabled all the time and received good food and bedding. Had these experiments been conducted under "veld conditions" the results may have been somewhat different; of the control sheep injected with the virulent strain more would probably have died, and amongst the immunized sheep tested with the same strain there may have been more showing clinical symptoms. Nevertheless, these experiments have yielded valuable results and have explained and corroborated much of the evidence which has accumulated during the 20 odd years the bluetongue vaccine has been in use.

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Studies on the Virus of Bluetongue.

By P. J. DU TOIT, B.A., Dr.Phil., Dr.Med.Vet., Director of Veterinary Services.

In 1905 Theiler showed that bluetongue was caused by an ultravisible, filtrable virus. He and other contemporary investigators (Spreull, Robertson, etc.) studied and described various other properties of the virus, so that the main characteristics of this virus have been known for more than 20 years.

In the course of immunization experiments recorded elsewhere in this volume, various points in connection with the virus of bluetongue again came under investigation, and the results obtained will be described here.

I. ATTENUATION OF THE VIRUS.

In his Annual Report for the year 1906-1907 Theiler gave a description of an experiment which led to the production of a vaccine against bluetongue. He showed that by simply sub-inoculating the virus from one generation of sheep to another, the mortality steadily decreased. From the first to the tenth generation the mortality was 11 per cent., and from the 11th to the 18th, nil.

This attenuated virus or vaccine was thereupon (in February, 1907) tried in practice and during the first year the mortality due to vaccination was 0.4 per cent., and subsequent relapses accounted for another 0.1 per cent. of deaths. During the same season and in the same districts where the vaccine had been tried, the mortality from natural bluetongue in non-vaccinated flocks amounted to 11 per cent.

Since then the vaccine has been used on a large scale and up to the end of 1928 more than 25 million doses had been issued.

The vaccine issued during all these years was made from the same strain which was used in Theiler's experiment in 1906. Last year the strain had reached the 54th generation.

It has been felt for some years that there was a distinct danger that the vaccine might become weaker and weaker and ultimately lose its power of protecting sheep. This possibility seemed all the more likely since the attenuation of the originally virulent strain proceeded so rapidly; within ten generations the virus had lost its power to kill. In view of this rapid attenuation, it seemed rather remarkable that the vaccine should have remained so constant during all those years.

Although the vaccine has continued to give satisfactory results, it was considered advisable to start a new strain, and to attempt the attenuation in the same way as that which led to so satisfactory a result in 1906.

The strain selected was obtained from a natural fatal case of bluetongue which occurred in a lamb (No. 16016) at Onderstepoort in February, 1927.

EXPERIMENT. (S. 2992.)

Object.—To attenuate a virulent strain of bluetongue virus with the object of producing bluetongue vaccine with the attenuated virus.

Method.—The strain was simply passed through a large number of generations of sheep. Usually two sheep were used in each generation and from the better reactor of the two, two further sheep were inoculated. The subcutaneous method of inoculation was employed and the dose given was 1 c.c.

Results.—For convenience the results may be tabulated in the following way:—

Generation	Date of Sub-inoculation.	No. of Sheep.	Temperature Reactions.	Deaths.
1	—	Lamb 16016	Positive.........	Died of bluetongue.
2	25/2/1927	2 Sheep.	Both Positive....	One died of heart failure.
3	7/3/1927	2 ,,	,, ,,	Both died of bluetongue.
4	23/3/1927	2 ,,	,, ,,	No death.
5	4/4/1927	2 ,,	,, ,,	—
6	14/4/1927	2 ,,	,, ,,	—
7	10/5/1927	2 ,,	,, ,,	—
8	19/5/1927	2 ,,	,, ,,	—
9	28/5/1927	2 ,,	,, ,,	—
10	8/6/1927	2 ,,	,, ,,	—
11	20/6/1927	2 ,,	,, ,,	One died of bluetongue.
12	2/7/1927	4 ,,	All Positive.....	—
13	11/7/1927	4 ,,	,, ,,	—
14	26/7/1927	2 ,,	Both Positive....	—
15	30/7/1927	2 ,,	,, ,,	—
16	17/8/1927	1 ,,	Positive	—
17	26/8/1927	2 ,,	Both Positive	One died of gangrenous proctitis.
18	7/9/1927	2 ,,	,, ,,	One died of heartwater.
19	17/9/1927	3 ,,	All Positive......	—
20	30/9/1927	4 ,,	3 Positive.......	One died of worm infection.
21	26/10/1927	4 ,,	3 ,,	—
22	10/11/1927	4 ,,	All Positive......	—
23	21/11/1927	4 ,,	,, ,,	One died of heartwater.
24	30/11/1927	2 ,,	Both Positive....	—
25	9/12/1927	2 ,,	,, ,,	—
26	18/12/1927	2 ,,	,, ,,	—
27	27/12/1927	2 ,,	,, ,,	—
28	5/1/1928	2 ,,	,, ,,	—
29	14/1/1928	2 ,,	,, ,,	—
30	23/1/1928	2 ,,	,, ,,	—
31	1/2/1928	2 ,,	,, ,,	—
32	10/2/1928	2 ,,	,, ,,	—
33	18/2/1928	2 ,,	,, ,,	—
34	27/2/1928	2 ,,	,, ,,	—
35	7/3/1928	2 ,,	,, ,,	—
36	16/3/1928	2 ,,	,, ,,	—
37	24/3/1928	2 ,,	,, ,,	—
38	2/4/1928	2 ,,	,, ,,	—
39	12/4/1928	2 ,,	,, ,,	—
40	21/4/1928	2 ,,	,, ,,	—
41	30/4/1928	2 ,,	,, ,,	—

It will be observed that deaths from bluetongue occurred in the 1st, 3rd, and 11th generation. Whether the death of one of the sheep in the second generation on the fourth day after inoculation can be ascribed to bluetongue is doubtful. From the 12th to the 41st generation there was no mortality from bluetongue; the few deaths that occurred in the inoculated sheep were definitely due to other causes.

Conclusion.—The attenuation in this case proceeded in almost exactly the same way as in Theiler's experiment of 1906. There, the last death from bluetongue was recorded in the 10th generation, here in the 11th.

The number of sheep used in our experiment is too small to allow us to calculate the percentage mortality and compare it with the mortality in Theiler's experiment. Nevertheless, the four deaths from bluetongue out of 21 sheep used in the first 11 generations would seem to indicate that the virulence of the strain was not very different from that selected by Theiler, where 10 sheep died out of 93.

Immunity Test. (S. 3589.)

In order to get an indication whether this strain would be suitable for vaccine purposes, a batch of 25 sheep were inoculated with blood obtained from a sheep in the 33rd generation. The inoculated sheep all gave typical temperature reactions without any clinical symptoms. They were bled, in the usual way, on the 9th day after inoculation and a batch of vaccine (No. 582) was prepared from this blood.

Three months after bleeding, these 25 sheep were tested for immunity. About half of them were injected each with 1 c.c. of their own vaccine strain (Batch 582), and the other half with a fairly virulent strain (No. 16357). All the sheep remained negative, although the control sheep gave good reactions.

Conclusion.—We conclude that the attenuated strain conferred an immunity on these 25 sheep which remained "solid" for three months.

The results obtained in the two foregoing experiments seemed to be entirely satisfactory. The virus had been attenuated to such an extent that no mortality occurred after the 11th generation, and the immunity conferred by this attenuated strain was sufficiently strong. It was decided, therefore, to proceed with the preparation of vaccine from this strain.

The 41st generation of virus was used and 10 batches of vaccine (606-615) were prepared from it. Altogether 250 sheep were injected in the course of this preparation, with entirely satisfactory results. The temperature reactions were typical and not too severe, and no other clinical symptoms or deaths from bluetongue were observed in any of the sheep.

These 250 sheep yielded over 400,000 doses of bluetongue vaccine which were issued and used during the 1928-1929 season. No untoward results were reported, so that it would definitely seem that the new vaccine is as satisfactory as the old vaccine had been during the last 20 years.

II. DILUTION AND PRESERVATION OF THE VIRUS (VACCINE).

In the course of Theiler's work on bluetongue, it was found that the virus, whether attenuated or not, could be preserved for a very long time in the following mixture:—

500 c.c. glycerine.
500 c.c. distilled water.
5 grams potassium oxalate.
5 grams carbolic acid.

This mixture is made up in bulk and filled into vessels, and the blood containing the virus is then added in equal quantity to this liquid. Hence the resulting mixture contains approximately 50 per cent. blood, 25 per cent. glycerine, ¼ per cent. potassium oxalate, and ¼ per cent. carbolic acid.

For the last 20 years the routine method of preparing bluetongue vaccine has been as follows: 700 c.c. of the above glycerine mixture is filled into fairly large Erlenmeyer flasks and the flasks sterilized. On the 9th day after inoculation the vaccine sheep are bled, and 700 c.c. blood is run direct from the jugular vein of each sheep into the 700 c.c. glycerine mixture. As stated above, this procedure has yielded entirely satisfactory results for the last 20 years. In a subsequent section it will be shown that the virus will "keep" in this mixture for a dozen years and more.

In 1925 the question was first raised whether the vaccine sheep could not be utilized to greater economic advantage. This could be done either by reducing the dose of vaccine or by diluting the blood further and keeping the dose per sheep at 1 c.c.

The former alternative was rejected, because it has been found by experience that the ordinary farmer cannot inject a quantity smaller than 1 c.c. with any degree of accuracy. Hence the second alternative was tried and a number of experiments carried out.

Object of the Experiments.—To determine whether the blood of bluetongue vaccine sheep which has been diluted more than 50 per cent. will be satisfactory for vaccine purposes.

Method of Experimentation.—In the initial tests the glycerine mixture described above was used, and blood added to it in the proportion of one part of blood to two parts or even three parts of glycerine mixture. This meant that if 700 c.c. of blood was taken from one sheep the final mixture would contain 2,100 or 2,800 c.c. vaccine, instead of 1,400 c.c. as was previously the case.

EXPERIMENT 1. (S. 2548.)

According to this method small experimental quantities of vaccine were prepared as follows:—

Batch 13802, dilution 1:2, October, 1925.
Batch 457, dilution 1:3, September, 1925.

This vaccine was tested in May, 1926, when batch 13,802 was seven months and batch 457 was eight months old.

Result.—Batch 13802 was injected into 10 sheep. Of these only three gave good temperature reactions, whereas the other seven remained negative.

Batch 457 was also tested on 10 sheep. Six reacted and four remained negative.

Conclusion.—It was thought at the time that the sheep used in this experiment may have been partly immune against bluetongue, and therefore the comparatively small percentage of reactors was not regarded as very significant.

IMMUNITY TEST. (S. 2580.)

At the conclusion of the above experiment the immunity of these 20 sheep was tested with the ordinary vaccine strain (batch 12773, prepared in October, 1925). One c.c. of vaccine was injected into each of these sheep as well as into five controls.

Result.—None of the 20 immunized sheep reacted, and of the five control sheep two reacted and three remained negative.

Conclusion.—This result was again unsatisfactory. The fact that only two out of five control sheep reacted was again explained on the assumption that some of the available sheep were immune against bluetongue, but in the light of subsequent results this assumption may not have been entirely justified.

Nevertheless the conclusion was drawn from the above results that the diluted vaccine (1:2 and 1:3) was active after seven and eight months respectively and that it had conferred immunity on the sheep.

Accordingly more vaccine was prepared in this way and issued for use in the field during the 1926-1927 bluetongue season.

The results were not entirely satisfactory and various complaints were received of cases where the vaccine apparently failed to give sufficient immunity.

In looking for an explanation for these failures, the fact was considered that the diluted vaccine contained a higher percentage of glycerine (and carbolic acid) than the original mixture. In the vaccine, as originally prepared, the percentages were roughly as follows:—

Blood	50 per cent.
Glycerine	25 per cent.
Carbolic acid	$\frac{1}{4}$ per cent.
Water	25 per cent.

Whereas, in the dilution 1:2 the percentages work out as follows:—

Blood	33 per cent.
Glycerine	33 per cent.
Carbolic acid	$\frac{1}{3}$ per cent.
Water	33 per cent.

and in the dilution 1:3:—

Blood	25 per cent.
Glycerine	37 per cent.
Carbolic acid	$\frac{3}{8}$ per cent.
Water	37 per cent.

It seemed not unlikely that the higher percentage of glycerine (33 or 37 as compared with 25) or possibly of carbolic acid ($\frac{1}{3}$rd or $\frac{3}{8}$ths as compared with $\frac{1}{4}$) would have a deleterious effect on the vaccine, either as regards its keeping qualities or its immunizing properties. To test this point a further experiment was undertaken.

EXPERIMENT 2. (S. 3018.)

Object.—To determine the effect, if any, of the concentration of glycerine and carbolic acid on the keeping and immunizing properties of bluetongue vaccine.

Method.—Five groups of sheep were injected as follows:—

(1) Four sheep with vaccine 1:3, age 1 day.
(2) Five sheep with vaccine 1:3, age $5\frac{1}{2}$ months.
(3) Five sheep with vaccine 1:3, age 7 months.
(4) Five sheep with vaccine 1:2, age 7 months.
(5) Five sheep with vaccine 1:1, age 17 months.

Result.—The five groups reacted as follows:—

(1) All four sheep positive.
(2) Two good reactions, two slight reactions, and one doubtful.
(3) One slight reaction, the other four very doubtful.
(4) One slight reaction, the other four doubtful.
(5) All five good reactions.

Conclusion.—Only the mixture 1:1 and the freshly prepared dilution 1:3 gave reliable results. Of the diluted mixtures the five and a half months old dilution of 1:3 gave the best results. The same dilution when seven months old seemed to be very much weaker. Also the dilution 1:2 appeared to be very weak after seven months.

The immunity of all these sheep was thereupon tested.

EXPERIMENT 3. (S. 3083.)

Object.—To test the immunity of sheep which, in Experiment 2, had been injected with various dilutions of vaccine.

Method.—For this test a fairly virulent strain of bluetongue virus (16188) was used, and every sheep received 1 c.c. subcutaneously. Two control sheep were added.

Results.—For convenience the results of Experiments 2 and 3 have been summarized in tabular form:—

Sheep No.	Experiment 2. Injected with.				Experiment 3. Immunity Test.	
	Vaccine.	Dilution	Age.	Result.	Virus.	Result.
16191	510	1 : 3	1 day	Slight reaction	16188	Negative.
16192	510	1 : 3	1 day	Good reaction	16188	Negative.
16193	510	1 : 3	1 day	Slight reaction	16188	Negative.
16194	510	1 : 3	1 day	Slight reaction	16188	Negative.

Sheep No.	Experiment 2. Injected with.				Experiment 3. Immunity Test.	
	Vaccine.	Dilution.	Age.	Result.	Virus.	Result.
16346	496	1 : 3	5½ months	Good reaction....	16188	Doubtful reaction.
16347	496	1 : 3	5½ months	Doubtful reaction.	16188	Good reaction.
16348	496	1 : 3	5½ months	Slight reaction...	16188	Doubtful reaction.
16349	496	1 : 3	5½ months	Good reaction....	16188	Negative.
16350	496	1 : 3	5½ months	Slight reaction...	16188	Negative.
16341	479	1 : 3	7 months..	Doubtful reaction	16188	Good reaction.
16342	479	1 : 3	7 months..	Doubtful reaction	16188	Good reaction.
16343	479	1 : 3	7 months..	Slight reaction...	16188	Good reaction.
16344	479	1 : 3	7 months..	Doubtful reaction	16188	Good reaction.
16345	479	1 : 3	7 months..	Doubtful reaction	16188	Good reaction.
16336	476	1 : 2	7 months..	Slight reaction...	16188	Good reaction.
16337	476	1 : 2	7 months..	Doubtful reaction	16188	Negative.
16338	476	1 : 2	7 months..	Doubtful reaction	16188	Good reaction.
16339	476	1 : 2	7 months..	Doubtful reaction	16188	Good reaction.
16340	476	1 : 2	7 months..	Doubtful reaction	16188	Negative.
16331	475	1 : 1	17 months	Good reaction....	16188	Negative.
16332	475	1 : 1	17 months	Good reaction....	16188	Negative.
16333	475	1 : 1	17 months	Good reaction....	16188	Negative.
16334	475	1 : 1	17 months	Good reaction....	16188	Negative.
16335	475	1 : 1	17 months	Good reaction....	16188	Negative.
16185	Control	—	—	—	16188	Good reaction.
16186	Control	—	—	—	16188	Good reaction.

Conclusions.—The above table shows clearly that the dilutions 1:3 and 1:2, when kept for a period of seven months, failed in the majority of cases, to protect sheep against bluetongue. Of the 10 sheep injected with such vaccine, only two proved to be immune when tested with a virulent strain.

Diluted vaccine (1:3) when kept for five and a half months, was found to be somewhat better. Four out of the five sheep were immune; only one which gave a doubtful reaction when vaccinated, reacted again when tested with the virulent strain.

The fact that the freshly prepared dilution 1:3 protected all the sheep shows clearly that the failure of so many of the sheep, which were vaccinated with the older dilutions, to react, is not due to the smaller quantity of blood which those sheep received, but rather to a deterioration of the virus when kept in that mixture.

The dilution 1:1, even though it had been kept for 17 months protected all five sheep. This is in accordance with the experience of 20 years.

The two control sheep reacted normally.

From these results we may conclude that *bluetongue vaccine when prepared according to the prescription given on page 82 for the dilutions* 1:2 *and* 1:3 *will deteriorate within six months and will fail to immunize sheep.*

As soon as the above information had been obtained from the experiments, the method of preparing the bluetongue vaccine was altered.

The dilution of the blood (one part blood to two parts of other ingredients) was maintained for reasons of economy, but the prescription was so altered that the final mixture contained the same percentage of glycerine, carbolic acid, and potassium oxalate as in the original prescription. In other words, only the amount of water was increased.

The prescription, then, which was used for the preparation of the vaccine in 1927 and 1928 reads as follows:—

Blood	33 per cent.
Glycerine	25 per cent.
Water	41 per cent.
Potassium oxalate ...	$\frac{1}{4}$ per cent.
Carbolic acid	$\frac{1}{4}$ per cent.

In making up the glycerine mixture in bulk, the same relative quantities of the ingredients are taken as was done previously, viz.:—

Glycerine	500 c.c.
Potassium oxalate	5 grams
Carbolic acid	5 grams

but, instead of adding 500 c.c. water, 833 c.c. are added.

In preparing the vaccine 1,400 c.c. of this mixture are filled into the Erlenmeyer flasks and 700 c.c. blood then added.

Vaccine prepared in this way has been tested after 16 *months* (*S.* 3644) *and found to be entirely satisfactory.*

An *immunity test* was also carried out with sheep vaccinated with such diluted vaccine (Exp. S. 2548). Twenty sheep were vaccinated and tested three months later with a virulent strain. Not one of the sheep reacted although the control sheep gave good reactions. The immunity was thus "solid" after three months.

During the 1927-28 and 1928-29 bluetongue seasons more than five million doses of vaccine prepared in this way, have been issued, and the results have been uniformly good.

We have, therefore, every justification to conclude that this alteration in the composition has not detracted from the value of the vaccine.

That this change has resulted in great economy, is obvious. Previously only 1,400 c.c. of vaccine was obtained from one sheep, whereas we now obtain 2,100 c.c.

III. RESISTANCE OF THE VIRUS (VACCINE).

Bluetongue has this property in common with horse-sickness, that its virus is extremely resistant. Even in decomposed blood the virus will remain active for a long period (one to two years) and in the glycerine-potassium-oxalate-carbolic acid mixture it will keep for several years.

In June, 1928, some unused bluetongue vaccine was returned to the Laboratory by the Government Veterinary Officer of Dundee, Natal. The vaccine had been kept in an ordinary office, with no cold storage or other facilities. Among this vaccine was one bottle which had been issued on the 19th October, 1917, and another issued on the 22nd December, 1909. The former bottle was therefore, about 10½ years old, the latter about 18½ years.

It was considered of sufficient interest to carry out a test with this old vaccine, to determine whether the virus was still active or not.

EXPERIMENT 1. (S. 3526.)

Object. To determine whether vaccine that had been kept for 10½ years was still active.

Method.—The usual dose of 1 c.c. was injected subcutaneously into three susceptible sheep.

Result.—One sheep gave a good temperature reaction, the second a slight reaction, and the third a somewhat doubtful reaction.

These three sheep were thereupon tested for immunity with a fairly virulent strain of bluetongue virus. The first two sheep remained negative while the third reacted.

Conclusion.—The 10½ years old vaccine produced a reaction on two out of three sheep and these two proved to be immune when tested afterwards.

EXPERIMENT 2.

Object.—To determine whether vaccine which had been kept for 18½ years was still active.

Method.—The vaccine was again injected into susceptible sheep. In view of the greater interest attached to this very old vaccine and the doubtful nature of some of the reactions, a larger number of sheep was used. In many cases sub-inoculations were again made from these sheep into further susceptible sheep; and in the majority of cases the immunity of the sheep was tested afterwards.

Results.—For greater convenience the results are again given in tabular form. Those of Experiment 1 have been included.

Exp.	Age of Vaccine.	Injected into Sheep No.	Blood sub-inoculated into Sheep No.	Result.	Immunity Test.	
					Virus	Result.
1	10½ years	21506	—	Reaction.........	20002	Negative.
		21474	—	Slight reaction...	20002	Negative.
		21496	—	Doubtful reaction	20002	Positive.
2	18½ years	19144	—	Reaction.........	16357	Reaction.
		19278		Doubtful mild reaction	16357	Negative.
		19320	—	Reaction.........	16357	Negative.

Exp.	Age of Vaccine.	Injected into Sheep No.	Blood sub-inoculated into Sheep No.	Result.	Immunity Test.	
					Virus.	Result.
		20954	—	Slight reaction...	20999	Late temperature reaction.
		—	20988	Probably negative	—	—
		20961	—	Slight reaction...	—	—
		—	21002	Slight reaction...	—	—
		20975	—	Slight reaction...	—	—
		—	20989	Reaction.........	—	—
		21456	—	Slight reaction...	20999	Slight reaction.
		—	21070	Slight reaction...	20999	Reaction.
		—	21082	Late reaction....	20999	Reaction.
		21473	—	Doubtful mild reaction	20999	Reaction.
		21475	—	Doubtful mild reaction	20999	Reaction.
		21494	—	Slight reaction...	20999	Slight reaction.
		—	21065	Probably negative	20999	Reaction.
		—	21097	Late reaction....	20999	Reaction.
		21560	—	Reaction.........	20999	Reaction.
		—	21114	No reaction......	20999	Reaction.
		—	21141	No reaction......	20999	Reaction.

It will be seen that altogether 11 sheep were injected with the 18½ years old vaccine. Of these eight gave definite reactions; and only in three cases were the reactions rather doubtful. Where sub-inoculations were carried out the diagnosis could, in the majority of cases, be confirmed, although in some cases (see, for instance, sheep 21560, which was sub-inoculated into 21114 and 21141) the results differed.

It will also be noticed that almost in every case where an immunity test was carried out, a positive reaction was obtained, even though the sheep might have given a reaction with the old vaccine previously. This was not to be wondered at. We know that the immunity against bluetongue is not absolute, and that it can be broken down either by using large quantities of the same virus or by using a different strain of virus. Here the sheep were first treated with an old, obviously weakened, vaccine, and then with a rather virulent strain of virus. It was only to be expected therefore, that these sheep would react again to the latter virus.

Comparing the results of Experiment 1 and 2, one gains the impression that the reactions obtained in the former were more definite, and that the immunity produced by the 10½ years old vaccine was stronger than in Experiment 2.

Conclusion.—Bluetongue vaccine which had been kept for 18½ years was still able to produce reactions in the majority of sheep, although the immunity conferred by this vaccine seemed to be very weak.

IV. PERSISTENCE OF THE VIRUS IN RECOVERED ANIMALS.

In the course of Theiler's work on bluetongue the question often arose, *how does the virus survive from one season to the next?* If the disease is insect-borne, as we believe it is, *where do the insects find the infection at the beginning of a new season?*

The "bluetongue season" is usually very limited and corresponds, generally speaking, with the rainy season. Cases of bluetongue are seldom, if ever, encountered before October or November, and the disease disappears in April, rarely as late as May. The difficulty is to bridge over the period May to October.

There is the possibility of the infected transmitting agent hibernating during this period; but most of the mosquitoes upon which suspicion rests, are shortlived, and there is no evidence in favour of such a view.

If sheep had been chronic carriers of the disease there would have been no difficulty in explaining the reappearance of the disease at the beginning of the next season, but this is not the case. Theiler (1909) came to the conclusion that "*immune animals do not retain the infection in the blood.*"

An experiment was undertaken to determine just how long after recovery the virus could still be demonstrated in the blood.

EXPERIMENT 1. (S. 3120.)

Method.—A sheep was selected (17446) which had given a good temperature reaction, and blood was taken from this sheep at intervals after recovery, and injected into susceptible sheep, in order to determine for how long such sub-inoculations would produce bluetongue reactions.

Results.—Sheep 17446 showed a temperature reaction which lasted from the 4th to the 15th day after inoculation.

Five days after recovery two sheep were injected with blood from 17446; both gave good reactions.

Twelve days after recovery one sheep was injected, which gave a mild reaction.

Eighteen days after recovery two sheep were injected; both gave mild reactions.

Twenty-five days after recovery two further sheep were inoculated. In both cases there was a slight irregularity in the temperature which went up to about 104° F., but it was somewhat doubtful whether this could be regarded as a bluetongue reaction.

The experiment was then terminated and a new experiment started.

Conclusion.—In the case of sheep 17446 the virus seems to have disappeared about three weeks after recovery from bluetongue.

EXPERIMENT 2. (S. 3194.)

Object.—Again to see how long after recovery the virus of bluetongue can be demonstrated in the blood.

Method.—Two sheep (18277 and 18285) were selected which had reacted rather severely to bluetongue, and their blood sub-inoculated into susceptible sheep at intervals.

Results.—For convenience the results of this experiment will be given in tabular form:—

Sheep No.	Period of Reaction.	Blood Sub-inoculated.		Result.	Remarks.
		Period after Recovery.	Into Sheep No.		
18277	5th–13th days after inoculation	4 days..	18285	Good reaction....	This sheep was also tested (see below).
		11 days.	18286	Good reaction....	—
		11 days.	18287	Good reaction....	—
		18 days.	18018	Late reaction....	—
		18 days.	18022	Late reaction....	—
		25 days.	18293	Late reaction....	—
		25 days	18297	App. negative....	—
		32 days.	18016	App. negative....	—
		32 days.	18295	App. negative....	—
		39 days.	18312	App. negative....	—
		39 days.	18320	Doubtful reaction	—
		58 days.	18303	Good reaction....	—
		58 days.	18306	Negative.........	—
		66 days.	18314	Good reaction....	—
		66 days.	18319	Slight reaction...	—
		78 days.	18335	Doubtful reaction	—
		78 days.	18726	Probably negative	—
		87 days.	18729	Irregular temp. reaction	Reacted again on being tested for immunity
		87 days.	18730	Probably negative	Reacted again on being tested for immunity
		109 days	18751	Late reaction....	Slight reaction on being tested for immunity
		109 days	18753	Late reaction....	Slight reaction on being tested for immunity.

Sheep No.	Period of Reaction.	Blood Sub-inoculated.		Result.	Remarks.
		Period after Recovery.	Into Sheep No.		
		129 days	18766	Slight reaction...	Slight reaction on being tested for immunity.
		129 days	18767	Doubtful reaction	Good reaction on being tested for immunity.
		149 days	19208	Negative.........	Good reaction on being tested for immunity.
		149 days	19242	Negative.........	Doubtful reaction on being tested.
18285	4th–11th days	4 days..	18290	Good reaction....	—
		4 days..	18291	Good reaction....	—
		11 days.	18298	Died of peritonitis	—
		11 days	18299	Reaction.........	—
		18 days.	18300	Reaction.........	Sub-inoculation also produced reaction.
		18 days.	18304	Reaction.........	—
		25 days.	18321	Late reaction....	—
		25 days.	18326	Late reaction....	—
		32 days.	18021	Late reaction....	—
		32 days.	18289	Late reaction....	—
		44 days.	18307	Good reaction....	—
		44 days.	18317	Reaction.........	—
		52 days.	18311	Probably negative	—
		52 days.	18313	Slight reaction...	—
		64 days.	18324	Probably negative	—
		64 days.	18333	Negative.........	—
		73 days.	18731	Reaction.........	No reaction on being tested for immunity.
			18732	Late reaction....	Good reaction on being tested for immunity. Sub-inoculation from 18732 produced a reaction in one out of 2 sheep.
		95 days.	18754	Slight reaction...	Slight reaction on being tested for immunity.
		95 days.	18755	Slight reaction...	Slight reaction on being tested for immunity.
		115 days	18768	Slight reaction...	Reaction on being tested for immunity.
		115 days	18769	Slight reaction...	Sub-inoculations into 2 sheep also produced slight reactions.
		135 days	19209	Negative.........	Mild reaction on being tested for immunity.
			19218	Very late doubtful reaction	Good reaction on being tested for immunity.

These results are not altogether clear. The sub-inoculations from sheep 18277 produced positive reactions until about 25 days after its recovery. Then followed a period during which no reactions could be obtained; this period lasted till the 39th day. After that, on the 58th and 66th days, definite reactions were again obtained. The results obtained from the following sub-inoculations were again rather doubtful, but then, on the 109th and 129th days after the recovery of sheep 18277, its blood again produced fairly definite reactions in susceptible sheep. The fact that these sheep, on being tested for immunity, again reacted, does not prove that the first reactions were not bluetongue, since we know that the immunity in this disease is by no means absolute. The last sub-inoculation from sheep 18277 on the 149th day gave no reactions.

The results obtained with the blood of sheep 18285 were, on the whole, more definite than those just discussed. The sub-inoculations on the 4th, 11th, 18th, 25th, 32nd, and 44th days all gave positive results. Then followed a period (52nd and 64th days) during which no reactions, or only very slight reactions could be obtained; and then again for a fairly long period (73rd, 95th, and 115th days) very definite temperature reactions were produced. The last sub-inoculation on the 135th day failed to produce definite bluetongue reactions.

Discussion of Results.

From these results it would appear that the virus of bluetongue may be present in the blood of recovered sheep for a fairly long period—say about four months. It would also appear that during this period there may be intervals during which the virus cannot be demonstrated in the blood. In the case of sheep 18277 there were two such intervals, about one month and about three months after recovery; and in the case of sheep 18285 there was one interval after about two months.

If in Experiment 1 the sub-inoculations from sheep 17446 had been continued for a longer period, it is very likely that the virus would again have appeared at a later stage.

It is difficult to account for this periodicity of the virus in the blood, and it would be futile to speculate on the probable causes of the phenomenon. However, the interesting fact remains that the virus does reappear in the blood after an absence. During the absence it must be present in some internal organ, or it may be in the blood itself, but in such small quantity that the sub-inoculation of 1 c.c. blood fails to reproduce the disease. Should this latter explanation be correct, then it would be quite conceivable that the transmitting agent of bluetongue could infect itself by sucking such blood; and it is equally conceivable that infection and transmission may take place at a much later stage than the four months during which the virus could be demonstrated.

For various reasons the experiment had to terminate after four months. Had it continued longer, it is possible that the virus would again have been found in the blood, perhaps after a further negative phase. However that may be, the experiment has shown that the question asked at the beginning of this chapter must probably be answered in this way, that *the virus of bluetongue survives in the* *recovered sheep* (perhaps only in a certain percentage), *and that the transmitting agent finds the infection here at the beginning of a new bluetongue season.*

Conclusion.—The virus of bluetongue may be present in the blood of recovered sheep for a period of four months, perhaps longer. During this period there may be intervals when the presence of the virus cannot be demonstrated by means of sub-inoculations into susceptible sheep.

SUMMARY OF CONCLUSIONS.

1. A virulent strain of bluetongue was attenuated by passage through sheep. After the 11th generation no more deaths occurred.

2. The strain was passed through 41 generations and a vaccine was then prepared which seemed to be entirely satisfactory.

3. For the preservation of the virus or vaccine, a mixture containing about 25 per cent. glycerine, $\frac{1}{4}$ per cent. potassium oxalate, and $\frac{1}{4}$ per cent. carbolic acid is used. If the percentage of these ingredients is increased, the vaccine will deteriorate and will not protect sheep against bluetongue.

4. The percentage of blood in the vaccine mixture has been reduced from 50 per cent. to 33 per cent., apparently with entirely satisfactory results.

5. Vaccine which had been kept for $18\frac{1}{2}$ years at ordinary room temperature was found to be active. It produced temperature reactions in the majority of sheep, although the immunity conferred by it seemed to be weak.

6. The virus of bluetongue may be present in the blood of recovered sheep for a period of about four months and perhaps longer. During this period there may be periods when the presence of the virus cannot be demonstrated.

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Section III.

Bacterial Diseases.

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E. M. Robinson	Outbreaks of Botulism in Domesticated Animals and Birds.
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Notes on Botulism in the Domesticated Animals.

By E. M. ROBINSON, M.R.C.V.S., Dr.Med.Vet., Senior Veterinary Research Officer.

DURING the past four years the writer has been engaged on research work in relationship to botulism of the domesticated animals. The present article is an interim summary of some of the more recent experiments carried out by the writer, several others being still in progress.

A fairly comprehensive paper by Theiler and Robinson (1927) summarizing our general knowledge of botulism in the domesticated animals, has recently been published. Appearing with the present article in this report of the Director of Veterinary Services, is a paper by Scheuber (1929) on the distribution of the spores of the lamsiekte organism (*Clostridium parabotulinum bovis*) on a lamsiekte farm. The article contains some interesting observations particularly in connection with the distribution in the soil and in the alimentary tracts of cattle.

The present article is divided into five sections dealing with different aspects of the subject. These are as follows, and will be discussed in order:

(1) Experiments with decomposed rats.
(2) Haemolytic action of toxins of the botulinus type.
(3) Passage of spores through animals.
(4) Absorption of toxins of the botulinus type by normal tissues.
(5) Anatoxin experiments.

(1) EXPERIMENTS WITH DECOMPOSED RATS.

In an article by Theiler and Robinson (1927) on equine parabotulism, mention is made of the part played by rats in the spread of the disease. In order to see to what extent rats were actually carriers of the infection, these animals were caught in different parts of the station and allowed to decompose in fruit jars. It was found that rubber rings were absolutely necessary on the jars, as however tight the lid of a jar without a ring was screwed down, larvae of flies, especially of the sarcophaga type could gain entrance. A point of interest, as it affects the incidence of botulism, is that during the coldest months, May to August, rats frequently would not decompose but tended to mummify. They could only be got to decompose by putting them into an incubator for a day or two at first, after which decomposition proceeded normally.

During a period from March, 1926, to November, 1927, 97 rats were allowed to decompose in this manner. Of these, seven proved toxic when material from their carcasses was tested on guineapigs. Two others proved toxic as well, but they had been experimentally dosed with cultures and then killed, so do not come under consideration. The rats which proved toxic were all killed in February and March, 1927. This toxicity during a limited period may explain the sporadic occurrence of equine parabotulism. In July, 1927, for instance, 24 rats were killed but none proved toxic. The number of rats is too small to draw any definite conclusions from, but can only give an indication.

The method of testing the decomposed rats was as follows: Fluid from a liquefying carcass after seven to ten days decomposition was given in a dose of 1 c.c. to guineapigs by the mouth. If the material proved toxic, it was subcultured in chopped meat medium and the strain kept for further study. It is of interest to note that the liquefaction fluid was in some cases toxic but only gave weakly toxic cultures in chopped meat medium. The flesh of the carcasses, however, invariably gave strongly toxic cultures. The explanation of this may be that the fluid contained only a few spores of the botulinus type which rapidly became suppressed by the growth of other bacteria.

Of the seven toxic rats it was only from three that cultures with a high toxicity were able to be maintained. The remaining four gradually lost their toxicity in subculture and were finally discarded. This has happened with a few strains of botulinus cultures isolated from different sources and the reason is apparently that the botulinus types can be suppressed by the associated bacteria until they finally disappear. The three toxic strains have been kept going in subcultures for about eighteen months without losing their toxicity in the least, and all three will usually kill guineapigs if inoculated subcutaneously with .001 c.c. of filtrate of any one of them.

In connection with this infection of rats, twenty-five from ninety rats killed in a raid on the forage store at Onderstepoort were opened and the stomach and intestinal contents sown in culture media. None of these cultures proved toxic subsequently.

It is uncertain how the carcasses of the rats which proved toxic got infected. Two sources are possible, one by the animal's eating food containing spores with invasion of the carcass after death from the intestine, or that the carcass is infected from the soil or by blowflies, etc., after death. In two rats dosed with cultures and killed the next day, the carcasses became toxic but the possibility of their having previously been infected was not excluded but was unlikely, as a number killed at the same time proved non-toxic. The toxins of the three rats referred to are highly toxic and for the purpose of reference will be given their serial numbers from the jars, i.e. three, five and six respectively.

By means of toxin-antitoxin tests, a comparison was made of the three toxins and an attempt to type them. The following antitoxins were available:

(1) Cl. botulinum A
(2) ,, ,, B
(3) ,, ,, A-B (Burroughs, Wellcome & Co.).
(4) ,, ,, (Rat 6) made by goat hyperimmunization.
(5) ,, ,, (D) or Cl. parabot. bovis do.
(6) ,, ,, (E) or Cl. parabot. equi do.
(7) ,, ,, (550) made from a toxin isolated from carcass material from Bechuanaland (a noted lamsiekte area).

Test 1.

Animal.	Toxin.	Antitoxin.	Result.
Guinea-pig	5 M.L.D.'s B. parab. (Seddon)....	3 c.c. Cl. bot. (rat 6)	Died in 48 hours.
,,	5 ,, Cl. ,, equi........	,, ,,	,, ,,
,,	5 ,, ,, botul. A..........	,, ,,	,, ,,
,,	5 ,, ,, ,, B..........	,, ,,	Died in 36 hours.
,,	2 ,, ,, parab. bovis......	,, ,,	Survived.
,,	5 ,, ,, botul. (rat 6)......	,, ,,	,,
,,	5 ,, ,, ,, (rat 5)......	,, ,,	Died in 48 hours.
,,	5 ,, ,, ,, (rat 3)......	,, ,,	,, ,,
,,	5 ,, ,, ,, (rat 6)......	No serum (control)	,, ,,

The results of this test are of considerable interest and would seem to show that the toxin of rat 6 and that of Cl. parabotulinum bovis were the same. Another point brought out is, that the toxin of rat 6 and the toxins of rats 5 and 3 were different. This latter point seemed rather unlikely and in view of the fact that the guineapigs from rats 3 and 5 died overnight without being seen during illness, it was decided to repeat the test with these toxins.

Test 2.

Animal.	Toxin.	Antitoxin.	Result.
Guinea-pig	5 M.L.D.'s Cl. parab. (rat 6).....	3 c.c. serum (rat 6)	Survived.
,,	5 ,, ,, (rat 3).....	,, ,,	,,
,,	1 ,, ,, (rat 3).....	,, ,,	,,
,,	5 ,, ,, (rat 5).....	,, ,,	Died in 48 hours.
,,	1 ,, ,, (rat 5).....	,, ,,	Survived.
,,	5 ,, ,, (rat 6).....	No serum (control)	Died in 72 hours.

From this test it would seem that the toxin of rat 5 varies to some degree from that of 6 and 3. The writer has come to the conclusion that of the types he has been dealing with in carcass material in South Africa all are closely related as shown by toxin antitoxin tests, but that small variations from one particular type occur. To confirm Test 2, it was repeated under very similar conditions with the same result.

Test 3.

Animal.	Toxin.	Antitoxin.	Result.
Guinea-pig	5 M.L.D.'s toxin (rat 6).........	3 c.c. (rat 6).......	Survived.
,,	5 ,, ,, (rat 3)..........	,, ,, ...	,,
,,	5 ,, ,, (rat 5)..........	,, ,, ...	Died in 72 hours.
,,	5 ,, ,, (rat 6)..........	Control (no serum).	Died in 48 hours.
,,	1 ,, ,, (rat 6)..........	,, ,,	Died in 6 days.

The results are essentially the same as those obtained in Test 2. An attempt was made to type the toxin of rat 5 and the results are given in the following test (number 4):

Test 4.

Animal.	Toxin.	Antitoxin.	Result.
Guinea-pig	5 M.L.D.'s (rat 5)..	3 c.c. Cl. parab. (550)...........	Survived.
,,	,, ,, ..	3 ,, ,, ,, equi. (E).......	Died in 72 hours.
,,	,, ,, ..	3 ,, ,, ,, bovis (D).......	Survived.
,,	,, ,, ..	1 ,, ,, botul. (A)............	Died in 72 hours.
,,	,, ,, ..	1 ,, ,, ,, (B).............	,, ,,
,,	,, ,, ..	Control (no serum).............	,, ,,

The antitoxins of both Cl. parabot. bovis (D) and of Cl. parabot. (550) neutralized the toxin of rat 5. The toxins of these two organisms, bovis and 550 are closely related, but as shown by some previous tests, apparently not identical. Toxin 550 being from an impure culture may be a mixture of more than one type of toxin.

Another test was carried out to see whether toxin from rat 6 could be neutralized by the antitoxins of Cl. parabot. bovis (D) and Cl. parabot. 550 respectively.

Test 5.

Animal.	Toxin.	Antitoxin.	Result.
Guinea-pig	5 M.L.D.'s (rat 6)..	3 c.c. Cl. parab. bovis (D).......	Survived.
,,	,, ,, ..	3 ,, ,, ,, (550)...........	,,
,,	,, ,, ..	Control (no serum)............	Died in 48 hours.

From this test it would appear that the toxins of Cl. parabot. bovis and 550 are very closely related to that of rat 6.

Conclusion.—One may summarize the experiments quoted in this section by stating that the carcasses of rats allowed to decompose at this laboratory may in a small percentage of cases become toxic. Material from them when dosed to guineapigs by the mouth produce typical symptoms of botulism. The toxins from three rats which were allowed to decompose were typed by toxin antitoxin tests and were shown to be closely related to if not identical with that of Cl. parabot. bovis, one of the bacteria responsible for the occurrence of " lamsiekte " in cattle.

The toxin of one rat, number 5, showed some variation from those of the other two but this variation was not sufficient to classify it as a different type.

(2) HAEMOLYTIC EFFECTS OF BOTULISMUS TOXINS.

In view of the fact that according to Ehrlich, tetanus toxin comprises two portions, a neurotoxin, tetanospasmin and a haemolysin, tetanolysin, the latter being haemolytic for the red corpuscles of various animal species, it was thought that botulismus toxins might have similar haemolytic effects.

Weinberg and Ginsbourg in their text book on anaerobes (1927), mention that the A and B types of botulinus organism are haemolytic in cultures 16-26 hours old but not later.

The botulismus toxins available for the experiment were of several types and were as follows:

(1) Cl. botulinum A (Lister Institute).
(2) Cl. botulinum B (Lister Institute).
(3) Cl. botulinum C (Graham & Boughton).
(4) Cl. parabotulinum bovis (Lamsiekte).
(5) Cl. parabotulinum equi (equine parabotulism).
(6) Bac. parabotulinus (Seddon).

The last three may be considered as of the C type, but Weinberg considers that one should call number (4) and (5) D and E respectively.

Toxic filtrates from all these different types were made from cultures in chopped meat medium after four days, incubation at 37° C. The toxicity of these filtrates was proved in each case before carrying out haemolysis tests as it has been found that one may encounter non-toxic strains of botulinus types. In the writer's experience these non-toxic strains produce no demonstrable toxin as guineapigs given large doses of filtrates from them subcutaneously fail to develop symptoms and subsequently are susceptible to a dose of toxin close to the minimum lethal. The haemolysis tests were carried out with washed red corpuscles of the horse, ox, sheep, pig, dog, rabbit and guineapig. The corpuscles were washed three times in normal saline, and for the tests 1 c.c. of a five per cent. suspension was used with varying amounts of the toxin. Of each toxin doses of 1 c.c. .5, .2 and .1 c.c. were tried and, in addition with sheep's corpuscles, fresh guineapig serum was added as well to act as complement.

In no case was any evidence of haemolysis obtained on incubation at 37° C. for 12 hours.

Conclusion.—One is entitled to conclude from the above experiments that no haemolysin is present in botulinus cultures of any of the known types except in the case of very young cultures.

(3) PASSAGE OF SPORES THROUGH THE ANIMAL BODY.

Various investigators have carried out experiments to determine whether toxin free bacteria of the botulinus type could produce toxin when inoculated into the living animal. These experiments are well summed up by K. F. Meyer (1928) in his article on "Botulismus" in the latest edition of Kolle and Wassermann's "Handbuch der pathogenen Mikro-organismen." He states that, whilst in moderate numbers no toxin is produced, when the bacteria are in enormous numbers, they can undoubtedly produce some toxin.

Scheuber (1928) has recently been able to obtain toxic cultures of the lamsiekte type from the faeces of cattle on a lamsiekte farm (Armoedsvlakte, Vryburg). These cattle were not actually suffering from the disease, and the method of making the cultures precluded the possibility of actual toxin having been introduced into the medium, the heating used after inoculation of the tubes being sufficient to destroy the toxin if it were present.

A point, however, which remained to be cleared up, was whether under certain conditions spores of botulinus types could vegetate, develop and produce toxin in the alimentary tract. It is conceivable that the organisms might find suitable conditions for development in some part of the alimentary canal.

In view of the fact that the Cl. botulinum A and B types sporulate more freely than the C species which includes the lamsiekte types, and that they can develop and produce toxin under conditions where the latter types cannot, it was decided to try the A type in the experimental work.

Experiments with many botulinus types here have shown that only the A and B grow with any freedom on vegetable media and produce toxin. Two fowls and two guineapigs were used for the experiments. Each was kept isolated in a cage which had been thoroughly cleaned. Before commencing the experiments, the faeces of each were cultured in chopped meat medium for the demonstration of botulinus spores and filtrates were made from the faeces. Both the cultures and the filtrates were tested on guineapigs in doses of 1 c.c. subcutaneously without any toxin being demonstrated.

The fowls and guineapigs were then dosed each with 2 c.c. of a culture of Cl. botulinum A in chopped meat after a week's incubation at 37° C. The culture was boiled for five minutes before dosing to ensure the toxin's being rendered inert. Temperatures of 80° C. for one hour have not always been found by the writer to be effective in killing the toxin as judged by subsequent guineapig inoculation.

After dosing, the faeces of each fowl and guineapig were collected daily for seven days commencing the day after the dosing. Cultures were made from the faeces in chopped meat medium every day and filtrates were made from the emulsions of the faeces. These filtrates were given subcutaneously to guineapigs in doses of 1 c.c. From both the fowls and guineapigs in each case the presence of botulinus spores could be traced in the faeces even after a week, but in decreasing numbers as judged by the toxicity of the cultures.

Larger animals such as cattle and sheep were not used for similar experiments, as though it would be easy enough to trace spores in the faeces if in fair numbers, the bulk of the faeces especially in cattle militates against testing for toxin. Unless the toxin were present in large amounts, it would not be traced, whereas in fowls and guineapigs one can deal with the greater part of the daily output of faeces.

No toxicity was demonstrated in any sample of filtrate from the faeces of the fowls or guineapigs during the seven days of the experiment, a finding which supports the view that botulinus types do not form toxin in the alimentary tract.

The writer has in two outbreaks of botulism in equines been able to demonstrate botulinus type spores in the caecal contents of horses and mules which died showing symptoms of paralysis. The observations in this connection are mentioned in another paper in this Report called "Notes on a few outbreaks of botulism in domesticated animals and birds."

Conclusion.—It would seem to be fairly certain that the bacteria of the botulinus type are not able to produce toxin in the alimentary tract of guineapigs or fowls, and it is probable that they do not find suitable conditions for development there.

(4) ABSORPTION OF BOTULINUS TOXINS BY NORMAL TISSUES.

During their work on tetanus toxin, Wassermann and Takaki were able to show that normal nerve tissues of animals were able to absorb it. They found that 1 gram of brain tissue for instance would absorb 10 M.L.D.'s of the toxin. Kempner and Schepilewski (quoted from K. F. Meyer, 1928) found that brain tissue would absorb botulinus A type toxin.

Having a number of different botulinus type toxins available, the writer attempted to repeat the experiments which had previously been done. Using guineapig tissues, absorption experiments were carried out with botulinus toxins and brain, liver, kidney and muscle.

About three to five grams of the tissue were ground up fine and suspended in 10 c.c. of normal saline. To the suspension an amount of toxin was added, varying from 10 M.L.D.'s down to as low as two or three. The potency of the toxin was always previously tested out on guineapigs and the minimum lethal dose may be taken as fairly accurate. The mixture was allowed to stand at room temperature for three hours, after which it was centrifugalized and the whole of the supernatant fluid inoculated into a guineapig. Room temperature means, between September and April, from 20 to 28° C. and between April and September with the central heating of the building, 16 to 20°C.

The toxins used were as follows:—

(1) Cl. botulinum A (A 223 Lister Institute, London).
(2) B. parabotulinus of Seddon (A C type of Cl. botulinum).
(3) Cl. parabotulinum equi (Cl. botulinum E according to Weinberg).
(4) Cl. parabotulinum bovis (Cl. botulinum D according to Weinberg).
(5) Cl. botulinum (No. 6 Mixed culture from dead rat).
(6) Cl. botulinum (No. 550 Mixed culture from cattle carcass).

No. (5) was a toxin isolated from the carcass of a dead rat allowed to decompose in a fruit jar. It may be a mixture of more than one type of toxin, but at present it has only been determined that the antitoxins for A, B, or E toxins do not neutralize it.

No. (6) was a toxin isolated from cattle carcass material in a bad lamsiekte district. It has not yet been definitely typed but differs slightly from the Cl. parabotulinum bovis or D type.

EXPERIMENT 1.—Cl. parabotulinum equi toxin (E).

Tissue.	Amount of Toxin.	Result.
Brain...............	About 2 M.L.D.'s (·001 c.c.)............	Died in 8 days.
Liver...............	,, ,, ...	Died in 3 days.
Muscle..............	,, ,, ...	Died in 2 days.

EXPERIMENT 2.—Cl. parabotulinum equi toxin (E).

Tissue.	Amount of Toxin.	Result.
Brain...............	About 1 M.L.D. (·0005 c.c.)............	Died in 72 hours.
Liver...............	,, ,, ...	Died in 48 hours.
Muscle..............	,, ,, ...	Died in 24 hours.

EXPERIMENT 3.—Cl. parabotulinum equi toxin (E).

Tissue.	Amount of Toxin.	Result.
Brain...............	About 2 M.L.D.'s.......................	Died in 4 days.
Liver...............	,, ,, ...	Died in 3 days.
Kidney.............	,, ,, ...	Died in 3 days.

EXPERIMENT 4.—Cl. parabotulinum equi toxin (E).

Tissue.	Amount of Toxin.	Result.
Brain...............	5 M.L.D.'s............................	Died in 4 days.
Muscle..............	,, ...	Died in 2 days.
Liver...............	,, ...	Died in 3 days.

EXPERIMENT 5.—Cl. parabotulinum bovis (D).

Tissue.	Amount of Toxin.	Result.
Brain...............	5 M.L.D.'s (0·1 c.c.)...................	Died in 10 days.
Muscle..............	,, ,, ...	Died in 48 hours.
Liver...............	,, ,, ...	Died in 48 hours.

EXPERIMENT 6.—B. parabotulinus (Seddon) toxin.

Tissue.	Amount of Toxin.	Result.
Brain...............	5 M.L.D.'s.............................	Survived.
Muscle..............	,, ...	Died in 8 hours.
Liver...............	,, ...	Died in 4 days.

EXPERIMENT 8.—Cl. Botulinum A Toxin.

Tissue.	Amount of Toxin.	Result.
Brain...............	2 M.L.D.'s (·001 c.c.)..................	Died in 7 days.
Muscle..............	,, ,, ...	Died in 4 days.
Liver...............	,, ,, ...	Died in 4 days.

EXPERIMENT 7.—B. parabotulinus (Seddon).

Tissue.	Amount of Toxin.	Result.
Brain...............	5 M.L.D.'s.............................	Survived.
Muscle..............	,, ...	Died.
Liver...............	,, ...	Died.

EXPERIMENT 9.—Cl. parabotulinum (554).

Tissue.	Amount of Toxin.	Result.
Brain...............	10 M.L.D.'s............................	Died.
Muscle..............	,, ...	Died.
Liver...............	,, ...	Died.

EXPERIMENT 10.—Cl. parabotulinum (554).

Tissue.	Amount of Toxin.	Result.
Brain...............	5 M.L.D.'s..............................	Survived.
Muscle.............	,, ...	Died.
Liver...............	,, ...	Died.

EXPERIMENT 11.—Cl. parabotulinum (550).

Tissue.	Amount of Toxin.	Result.
Brain...............	5 M.L.D.'s..............................	Died.
Liver...............	,, ...	Died.
Kidney............	,, ...	Died.

SUMMARY.

The ability of brain tissue of a susceptible animal to absorb toxins of the botulinus type appears to be rather variable. The absorption does not in any case occur to a marked degree. With Cl. botulinum equi toxin there appeared to be very little absorption. With the other toxins the absorption was variable but 5 M.L.D.'s appear to be about as much as can be absorbed by three to five grams of brain tissue.

Cultures of the botulinus type made in Hibler's brain medium possess very little toxicity even when made from highly toxic cultures, whereas beef heart medium produces highly toxic cultures.

(5) NOTES ON ANATOXINS IN BOTULISM.

The application of formalin to the production of anatoxins for tetanus has been applied on a large scale by Ramon and co-workers (1925) and the method has been used for botulinus toxins by Weinberg and Goy (1925). In immunization against botulinus toxins A and B they found that the best results were obtained when they used moderate doses of anatoxin at regular intervals, and the immunity was better than from one large dose. When rabbits were immunized by giving them four injections at 12-14 day intervals of quantities of 5, 10, 15 and 20 c.c. of anatoxin produced by the addition of 2 per cent. formalin, the rabbits' serum in a quantity of 1 c.c. would neutralize 70,000 M.L.D.'s for the guineapig. Later, in a recent article, Salsbery and Jacobi (1928) have published some preliminary notes on anatoxins for botulinus types, and a further extensive article has been promised by Graham, but at the time of writing has not appeared in print.

The writer has carried out a number of experiments with anatoxins for various botulinus types. These experiments are still being carried out, but preliminary notes on those done so far may be of interest. The toxins available were those already mentioned in another part of this article (section on absorption of toxins by normal tissues). Before formalinization the approximate M.L.D. of each toxin was determined for future comparison. Formalin in a concentration of .2 up to 1 per cent. was used in different experiments, and the toxins were subsequently kept at 37° C. for varying periods of time.

A series of experiments was carried out to observe the effect of formalinization on the toxins and the results may be briefly tabulated. Guineapigs were used for testing the anatoxin and were inoculated subcutaneously.

EXPERIMENT 1.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
B. parabot. (550) M.L.D. (·005 c.c.)	·4 %	1 day.......	1 c.c.......	Died.
		3 days......	1 c.c......	Died.
		5 days......	1 c.c......	Survived.

In this case five days were necessary before the toxin was rendered atoxic.

EXPERIMENT 2.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
B. parabotul. (554), rat 6, M.L.D. (·001 c.c.)	·4 %	1 day.......	1 c.c......	Died.
		3 days......	1 c.c......	Died.
		5 days......	1 c.c......	Survived.

Again in this case five days were necessary before the toxin was rendered harmless.

EXPERIMENT 3.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
B. parabot. (554), rat 6, M.L.D. (·001 c.c.)	·4 %	4 days......	1 c.c......	Died.
		9 days......	1 c.c.......	Survived.
		14 days.....	1 c.c......	Survived.

With this toxin four days formalinization might fail to render it atoxic.

EXPERIMENT 4.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
Cl. botul. A., M.L.D. (·01 c.c.)	·4 %	1 day	1 c.c.	Died.
		3 days	1 c.c.	Survived.
		7 days	1 c.c.	Survived.

In this case three days was sufficient.

EXPERIMENT 5.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
Cl. botul. B., M.L.D. (·01 c.c.)	·4 %	36 hours	1 c.c.	Survived.
		3 days	1 c.c.	Survived.
		7 days	1 c.c.	Survived.

In this case 36 hours was sufficient.

EXPERIMENT 6.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
Cl. parabot. equi. (E), M.L.D. (·01 c.c.)	·4 %	36 hours	1 c.c.	Survived.
		3 days	1 c.c.	Survived.
		5 days	1 c.c.	Survived.

In this case 36 hours was sufficient.

EXPERIMENT 7.

Toxin and M.L.D.	Formalin.	Time at 37° C.	Dose.	Result.
Cl. parabot. (523). An impure toxin from carcass material, M.L.D. (·0001 c.c.)	·2 %	24 hours	1 c.c.	Died.
		3 days	1 c.c.	Died.
		5 days	1 c.c.	Died.
	·4 %	24 hours	1 c.c.	Died.
		3 days	1 c.c.	Died.
		5 days	1 c.c.	Died.
	1 %	24 hours	1 c.c.	Died.
		3 days	1 c.c.	Died.
		5 days	1 c.c.	Survived.

Only the guineapig receiving 1 c.c. of toxin to which 1 per cent. formalin had been added and incubated for five days survived.

Other experiments were carried out on similar lines using formalin with similar results. A point brought out very clearly was that the length of time necessary for the formalin to act was directly in proportion to the virulence of the toxin.

In Experiment 4 the two surviving guineapigs were inoculated 18 days after the first inoculation, with virulent toxin. One received 100 M.L.D. and the other ten. The former died but the latter survived.

In Experiment 3 the two survivors were inoculated 18 days later with virulent toxin. One received 100 M.L.D. and the other ten. In this case the latter only survived. Other experiments were carried out with guineapigs surviving anatoxin tests, and all showed that no very powerful immunity results in guineapigs from one inoculation of anatoxin, at least in a dose of 1 c.c.

In experiments on goats where they were given doses of 10 and 20 c.c. of anatoxin with a ten-day interval a strong immunity developed.

Goats and rabbits were hyperimmunized against various toxins using anatoxin as the initial immunizing agent. Only a few rabbits were done as the amount of serum to be obtained from them was insufficient for subsequent toxin-antitoxin tests.

Several goats were immunized against different toxins. Antitoxins were produced against the following toxins:—

(1) Cl. botulinum A (Lister Institute Strain).
(2) Cl. botulinum B (Lister Institute Strain).
(3) Cl. botulinum D (Cl. parabot. bovis).
(4) Cl. botulinum E (Cl. parabot. equi).
(5) Cl. botulinum 554 (Rat 6).
(6) Cl. botulinum 550 (carcass material, Bechuanaland).

The method of immunization was the same in each case. Commencing with a dose of 5 c.c. of a homologous anatoxin, a further dose of 10 c.c. was given a week later. Two weeks later a dose of 10 M.L.D.'s of virulent toxin was given subcutaneously and subsequently the dose was doubled every week until 2,000 M.L.D.'s were given. The doses from 100 M.L.D.'s onward were given intravenously, so as to avoid giving large doses of fluid under the skin.

The serum of the goats was tested at intervals during the immunization, and it was found that when 2,000 M.L.D.'s had been reached, the goats' serum in a dose of 1 c.c. would usually protect guineapigs against 200 to 500 M.L.D.'s of the particular toxin used to immunize the goat. The hyperimmunization was not carried further, as the sera were quite good enough for cross toxin-antitoxin tests. A toxic Bot. C. strain was not available, but will shortly be on hand, and it is the writer's intention to attempt a classification of all the known C types by serological tests.

The anatoxin method as shown by numerous authors, is the best available method for producing immunity against bacterial exotoxins of whatever type. A good immunity can be produced by anatoxin alone, but with subsequent inoculations of virulent toxin animals can easily and safely be rendered hyperimmune.

Prior to using anatoxins, the writer was only once able to produce a hyperimmune serum against any of the botulinus strains met with in South Africa. He found that at any stage in the earlier inoculations with virulent toxin an increase in the dose might produce fatal results. The method previously used by the writer was to commence with sublethal doses of toxin and continue past the minimal lethal dose by small increases. It was found in this method that a dose such as 5 M.L.D.'s would produce no symptoms but ten might, a week later, produce fatal symptoms.

At present there is no necessity to immunize against " lamsiekte " in cattle, as the use of bone meal has proved so effective in preventing it, but such an immunization could now be undertaken at any time. It would, however, be necessary before doing so to determine more accurately whether there is more than one toxin responsible as the experiments so far carried out would tend to show.

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Notes on a Few Outbreaks of Botulism in Domesticated Animals and Birds.

By E. M. ROBINSON, M.R.C.V.S., Dr.Med.Vet., Senior Research Officer.

SEVERAL outbreaks of botulism in equines have been investigated by the writer during the past two years, and some of these will be briefly described as they were of special interest. Since the publication of an article by Theiler and the writer (1927) many communications from veterinary surgeons and horse owners have been received quoting personal experience of outbreaks of paralysis in equines. These outbreaks were never realized at the time to be probably due to botulism, but there is little doubt that most of them were.

The first outbreak to be described occurred in a contractor's stables about three miles from Pretoria. There were 85 mules and six horses in the stables, and they were out at work all day except on Sundays when they were allowed to run in a large camp fenced in with barbed wire. The occurrence of the disease was reported by a practitioner in Pretoria, who stated that eight days previous to bringing the matter to our notice, a mule had developed symptoms of weakness of the limbs and had been found cast under the manger. On being lifted it was able to stand, but soon went down again and lay stretched out on its side. If propped up it could eat and showed no interference with mastication or deglutition. No temperature was shown during the illness. A second mule developed symptoms of an identical nature five days later, and a third the day before the writer saw the animals. The last case was standing up but was weak and staggered when walked. The temperature was 101° F., pulse 50, respiration normal. The animal was eating and drinking well.

The two first-mentioned mules were killed as they were *in extremis*, and post-mortems were made on them. No pathological changes were found in the animals, so caecal contents were taken from both animals for inoculation into culture media to attempt the demonstration of botulinus spores.

The animal which was left was brought to the laboratory for observation, but lived for only three days. At post-mortem again no pathological changes were seen. A further case occurred, making four in all. This animal was brought to the laboratory as well, but apparently was not as bad as the others and recovered in the course of three to four weeks. In none of the four cases was any interference with mastication or deglutition observed, but this symptom is chiefly seen in acute cases of botulism.

Attempts to trace the source of the infection failed. The day's supply of food was cut up at one time. It consisted of bales of oat forage and a ration of maize. No dead rats had been seen in the forage, but there was evidence of the presence of numerous rats in the forage store. The food supply could not definitely be incriminated, and in any case it was food supplied a week to a fortnight before, which had caused the outbreak. The four mules were all in different parts of a large stable, so that the infection had an uneven distribution. The camp where the animals were turned out showed nothing of a suspicious nature, and there was very little grazing.

The caecal contents from one mule, when cultivated in chopped meat medium, gave a toxin which produced typical symptoms of botulism in guineapigs when dosed to them by the mouth. The strain of botulinus organism was kept going in culture media for several months, but apparently the contaminating organisms were too vigorous and gradually the toxicity was lost. Unfortunately, at the time no variety of antitoxins was available to type it with, so the opportunity was lost. Botulinus antitoxin A and B (polyvalent) failed to protect against the toxin.

Having seen a reference to the ability of the serum of an animal with subacute botulism to neutralize small doses of toxin (Tocher, 1923), an attempt was made with serum of one of the living cases to see if the toxin could be typed. An experiment was carried out as follows:—

Aminal.	Toxin.	Serum of Mule.	Result.
Guineapig	*Cl. botulinum* A, 5 M.L.D.	5 c.c.	Died.
,,	,, ,, ,,	None	,,
,,	,, ,, B, ,,	5 c.c.	,,
,,	,, ,, ,,	None	,,
,,	,, ,, C, ,,	5 c.c.	,,
,,	,, ,, ,,	None	,,
,,	*Cl. parabot. bovis*, ,,	5 c.c.	,,
,,	,, ,, ,,	None	,,
,,	*Cl. parabot. equi* ,,	5 c.c.	,,
,,	,, ,, ,,	None	,,
,,	*B. parabotulinus* (Seddon), 5 M.L.D.	5 c.c.	,,
,,	,, ,, ,, ,,	None	,,

The mule's serum showed no neutralizing effect on any of the toxins. It is doubtful if any conclusion could be drawn from this experiment, as it is uncertain whether any definite amount of antitoxin is produced during one attack of botulism.

There is, of course, no proof that the spores found in the caecal content of the mule were associated with the disease, but the evidence in these outbreaks of botulism is always strong circumstantial only but usually convincing.

The second outbreak to be described occurred on a farm halfway between Johannesburg and Pretoria. The farmer came in to ask advice about the treatment of a horse which had suddenly developed paralysis and was unable to rise. He had lost a horse with similar symptoms a few days previously. Botulism was suspected, and on inquiry it was found that the farmer had been carrying out a campaign against rats in his stables and forage stores. He was requested to send in samples of the contents of the caecum and colon if the horse died, and this he did. Cultures made in chopped meat from the caecal contents became toxic and on dosing them to guineapigs per os, symptoms typical for botulism were produced. Here, again, one has an instance of cultivation of the probable causal arganism from intestinal contents, but the evidence is again circumstantial.

This strain has been maintained in culture media without any marked loss of toxicity and the minimum lethal dose for guineapigs subcutaneously was found to be in the neighbourhood of .001 c.c. Per os, .1 to .5 c.c. was the M.L.D.

Attempts were made to type this strain which was given its serial culture number 628. Several antitoxins were available, all made by hyperimmunization of goats except the polyvalent Botulinus A and B antitoxin which was obtained from Burroughs Wellcome & Co. In two series of comparative tests carried out the results were very similar.

TEST 1.

Animal.	Toxin.	Antitoxin.	Result.
Guineapig	628, ·01 c.c. subc.	3 c.c. *Cl. parab. boris*..........	Survived.
,,	,, ,,	3 ,, ,, *equi*	Died (? botulism).
,,	,, ,,	3 ,, ,, (554)..........	Died.
,,	,, ,,	3 ,, ,, (550)..........	Survived.
,,	,, ,,	1 ,, *Cl. botulinum* A..........	Died.
,,	,, ,,	1 ,, ,, A and B....	Died.
,,	,, ,,	No antitoxin..................	Died.

It was decided to repeat this test owing to the survival of two guineapigs given different antitoxins and one in which there was some doubt as to the cause of death. On repeating the test the guineapng with *Cl. parabot. boris* and 550 again survived.

The antitoxins used were as follows:—

(1) *Cl. botulinum* A (by goat hyperimmunization).
(2) *Cl. botulinum* B (by goat hyperimmunization).
(3) *Cl. botulinum* A-B (Burroughs Wellcome & Co.).
(4) *Cl. parabotulinum boris* (D according to Weinberg).
(5) *Cl. parabotulinum equi* (E according to Weinberg).
(6) *Cl. parabotulinum* (550) impure toxin from carcase material.
(7) *Cl. parabotulinum* (554) impure toxin from a rat carcase at Onderstepoort.

The latter two antitoxins were made in the attempt to type the toxins by cross toxin antitoxin tests with all available types. From the tests it would appear that toxin 628 belongs to the *Cl. parabot. boris* or D type. The type 550 is closely related to the D type, and the writer has a good deal of evidence to show that when doses of toxin close to the M.L.D. are used the antitoxin against either type may neutralize both toxins.

A third outbreak occurred in a brickyard in Johannesburg. It commenced with the death of the owner's horse, which was fed on lucerne and chopped oat forage. The animal was taken ill and died the following day with symptoms of complete paralysis. It ate until it became paralysed, and before it went down showed symptoms of abdominal pain, getting up and lying down continuously.

A few days later when the outbreak was investigated by the Government Veterinary Officer there were two donkeys showing symptoms, and a horse and a donkey which had just died.

In both the donkeys the temperature and pulse were normal, but otherwise the only symptoms were weakness and inability to rise when down. It was noticed that the forage store and stables were riddled with rat holes and excreta of rats were everywhere. Dead rats had been seen in the forage from time to time. On a search being made the partially mummified carcase of a kitten was found in the forage and was sent to the laboratory for investigational work.

On cultures being made from this carcase in chopped meat medium, a powerful toxin was produced. The symptoms produced when the toxic cultures were dosed to guineapigs per os were typical of botulism. The serial culture number was given to this toxin, i.e. 11, and an attempt was made to type it by toxin antitoxin tests. The antitoxins used were the same as were used with toxin 628. As will be seen from the table the results of the tests were regular.

TEST 3.

Animal.	Toxin.	Antitoxin.	Result.
Guineapig	·01 c.c. (11)	Subc. 3 c.c. *Cl. parab. bovis* (D).......	Survived.
,,	·01 ,, ,,	,, 3 ,, ,, ,, *equi* (E).......	Died in 72 hours.
,,	·01 ,, ,,	,, 3 ,, ,, ,, (554)..........	,, 72 ,,
,,	·01 ,, ,,	,, 3 ,, ,, ,, (550)..........	,, 96 ,,
,,	·01 ,, ,,	,, 1 ,, ,, *botulinum* A..........	,, 72 ,,
,,	·01 ,, ,,	,, 1 ,, ,, ,, A and B....	,, 72 ,,
,,	·01 ,, ,,	,, no antitoxin..................	,, 72 ,,

The M.L.D. for toxin (11) was found to be approximately .005 c.c. From this test one is entitled to conclude that the toxin (11) is the same as that of *C. parabotulinum bovis*, the organism described by the writer as the cause of lamsiekte in cattle in Bechuanaland. Since isolating the latter organism and carrying out comparative tests with other strains from the same area the writer has reason to believe that more than one type of the botulinus organism may exist there as shown by cross toxin antitoxin tests.

Very recently (December, 1928), an outbreak of what was undoubtedly botulism occurred amongst the birds in the National Zoological Gardens in Pretoria. A similar outbreak had occurred three years before, but was not investigated at the time. In this recent outbreak subsequent to very heavy rains, 18 water birds died in one of the ponds for aquatic species. These birds included Nile geese, grey-backed ducks and other species. The mortality occurred within a period of 24 hours and none has since died. In those birds which were seen alive the symptoms were fairly typical. There was great weakness, inability to stand, and the birds lay with the head stretched on the ground.

Post-mortem there was little in the way of lesions to be seen. In a few cases there was evidence of catarrhal gastro-enteritis, and in two some degree of hydropericardium.

Cultures were made in chopped meat media from the intestines and caeca of six of these birds. From two of these cases toxic cultures were obtained which, when dosed to guineapigs by the mouth, produced typical symptoms of botulism.

The evidence here as to botulism being the cause of the mortality in the birds is circumstantial. The writer was away at the time of the outbreak, so the intestinal contents were not tested for toxicity, but only cultures were made and kept for his return.

An examination by the writer was made of the pond where the ducks died, some two weeks after the cases occurred. All the birds which died were in a pond separated from another one by a narrow culvert, and the water flowed from the latter to former. No mortality occurred in the latter pond. The water in the ponds came from the town supply and first passed through a large ornamental pond for gold fish. It was difficult to incriminate the water supply and the possibility of dead fish had to be considered. From the pond where mortality occurred and which was well stocked with carp, four of the latter fish and a gold fish were obtained. The intestinal contents of the four carp were subcultured into chopped meat medium and the gold fish was allowed to decompose in a sealed fruit jar. Faeces from the birds in the pond were collected and subcultured in chopped meat medium.

When dosed to guineapigs subsequently, none of the cultures proved toxic, nor did the decomposed gold fish and cultures from the birds' faeces.

It is very probable that the botulism-producing factor was only present after the heavy rain, and such a factor might be earthworms, which only appear at the surface after heavy rains. The actual source of the botulinus organisms must, therefore, be regarded as undetermined in this outbreak.

In conclusion, it will be of interest to mention the occurrence of botulism in sheep in South Africa. In certain portions of the Western Province sheep suffer from what is apparently a phosphorus deficiency and develop a marked pica. They chew bones whenever they get the opportunity, and according to Mr. J. G. Bekker, Veterinary Research Officer, who reported the condition, the sheep have abnormally light bones which break on the least provocation. On this account it is often dangerous to catch them by the legs or throw them. Material from carcases sent in by Mr. Bekker was used for cultural work, and from portions of muscle, marrow and bone which was still moist, toxic cultures were obtained producing symptoms of botulism when dosed to guineapigs.

In these cases and those of birds in the Zoological Gardens, the type of botulinus organism responsible has not yet been determined. The cultures are only weakly toxic, and an attempt is being made to increase their toxicity before typing them.

There is no doubt that botulinus organisms are widely distributed in the soil in South Africa, and probably occur everywhere, though it is known that carcase material does not become toxic in some parts of the country. At the laboratory here some ten years ago carcases of cattle allowed to decompose in the open veld, did not become toxic unless the animals had died from lamsiekte (botulism) as a result of being dosed with toxic culture material by the mouth.

Cases of human botulism have not so far been recorded from South Africa. The writer has inquired of the Director of the Medical Research Institute in Johannesburg, the premier medical research laboratory in South Africa, as to whether he knew of any outbreaks of botulism in this country, or whether the A and B types had been isolated, but he was not able to refer me to any record of human botulism.

It is inconceivable when one considers the ubiquitous distribution of the A and B types that they should be absent from South Africa, more especially when one considers the wide distribution of the C types. The writer considers the types he has mentioned as D and E, to be very closely related to the American C type of Bengston (1923) and Graham and Boughton (1923).

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Fig. 1.

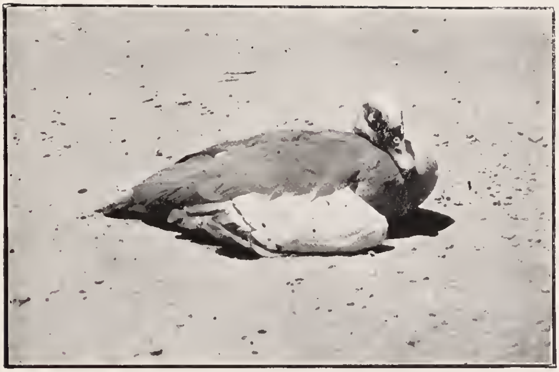
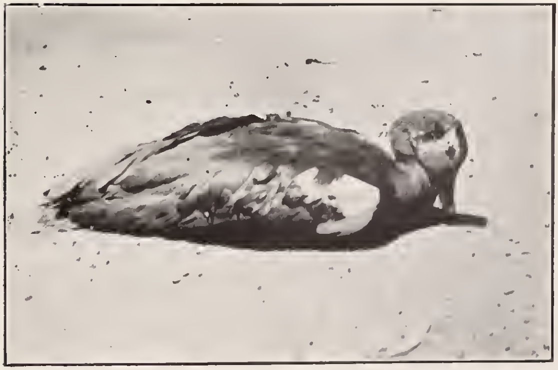


Fig. 2



Figs. 1 and 2.—Grey-backed ducks showing symptoms of botulism.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

A Note on *Corynebacterium ovis* (Bacillus of Preisz Nocard).

By E. M. ROBINSON, M.R.C.V.S., Dr.Med.Vet., Senior Research Officer.

In a previous article the writer (1928) described an icterus of sheep which could be produced at will by means of cultures of a particular bacterium. In a footnote to this article it was mentioned that evidence had later been obtained that this bacterium was closely related to, if not identical with, the Bacillus of pseudo-tuberculosis in sheep (B. Preisz Nocard). The purpose of the present article is to record the production of icterus in sheep by means of the Preisz-Nocard bacillus.

Carre and Bigoteau (1908) have recorded the fact that the inoculation of this organism into sheep might produce haemoglobinuria. Boquet (1912) described the haemolytic effect of the toxin of the organism on various types of red blood corpuscles. In this connection it may be of interest to mention that the writer (1928) was unable to demonstrate any haemolytic effect of saline suspensions of blood agar cultures of the organism of bacterial icterus on washed sheep's red corpuscles nor could the haemolysin be demonstrated in broth cultures.

The observations in the present note were made as the result of an attempt to produce cases of typical pseudo-tuberculosis in sheep experimentally for the purpose of demonstration to students. For this purpose two sheep were used, one inoculated intravenously and one subcutaneously under the skin of the thigh. The subsequent history of these two sheep is as follows. In each case the material used was a saline suspension of a blood agar culture four days old, using 10 c.c. of saline to a culture. (Strain used was isolated from the lung of a sheep six months previously.)

Sheep (lost number). Received 5 c.c. culture suspension intravenously. At the 24th hour after the inoculation the temperature had risen to 105.8° F. The animal showed all the symptoms of an acute septicaemia, i.e. loss of appetite, extreme dullness, etc., and it died 48 hours after inoculation.

At the post-mortem it was noticed with surprise that the case was one of marked general icterus, as the cause of death had been thought to be probably a simple bacterial septicaemia.

When opened, the sheep showed a very marked general icterus throughout, the fat being of a deep yellow colour. The liver was a deep chrome yellow in colour, and the kidneys were enlarged and showed a dark greenish-brown pigmentation in the cortex. The spleen was enlarged and the pulp swollen, soft and dark reddish-brown in colour. The case was obviously one of acute bacterial icterus identical with those produced by the organism of bacterial icterus.

Sheep 13851. Received 5 c.c. culture suspension subcutaneously. There was an initial rise of temperature to 105° F. on the third day, but it subsequently fell to normal to rise again to 105° F on the 7th and 15th days, after which it remained between 103° and 104° F. until the sheep was killed on the 40th day. Ten days after inoculation the lymphatic glands at the site of inoculation became enlarged and subsequently several nodular swellings formed, about the size of a pea up to that of a hazel-nut. These nodules were firm in consistence and gradually increased in size up to the time the animal was killed. From the third week after inoculation the sheep showed a very rapid loss of condition, so that at the time it was killed it was so weak that it could hardly stand. It was thought that probably there would be internal lesions due to the organism, but at the post-mortem these were not found.

One may briefly summarize the results of the post-mortem by saying that no pus formation of any kind was found except at the inoculation site. The nodules there each consisted of a firm greenish-yellow purulent material surrounded by a fibrous capsule. From this pus the organism was recovered in pure culture.

The carcase of the sheep showed emaciation with very marked anaemia, but otherwise there were no lesions. The result of this inoculation confirms the experience of other workers, that one may get a very marked emaciation in sheep as a result of the presence of lesions due to the Preisz Nocard organism, even when these lesions are not at all extensive.

In view of the results obtained from the inoculation of these two sheep, it was thought advisable to repeat the experiment but using intravenous inoculation only with varying doses.

Two sheep were inoculated intravenously with a saline suspension of a four-day-old blood agar culture of the Preisz Nocard organism with the following results.

Sheep 15756. Received 5 c.c. intravenously, the same dose as used for the previous sheep inoculated intravenously. Again in this case symptoms of an acute septicaemia supervened and death resulted in 36 hours. No haemoglobinuria was noticed and the visible mucous membranes were a deep reddish-brown just before death.

At post-mortem the lesions of icterus were not well developed as death occurred too soon after inoculation.

Post-Mortem Report (Sheep 15756).

Blood, uncoagulated, dark red.

Subcutis, fat with a yellow tinge.

Lungs, marked hyperaemia. Bronchi filled with yellowish foam.

Liver, enlarged. Chocolate brown colour. Uncoagulated dark red blood in the veins. *Bile*, dark green, fluid.

Spleen, pulp swollen, reddish black, soft in consistence.

Kidneys, cortex dark reddish-brown with a mottled appearance In the *coecum* and *colon* the faeces were dry and of a dark brown colour. *Bladder*, empty and contracted.

From this brief post-mortem report it will be seen that the sheep died of an acute toxaemia before marked lesions of icterus could develop.

Sheep 15755. Given 1 c.c. intravenously. A smaller dose was given in this case to see what results would be produced.

In this case the temperature rose to 104° F. at the 48th hour after inoculation. The sheep was seen to be dull, not eating and the respiration was rapid. Slight icterus was noticed. 24 hours later the temperature had risen to 105° F. On examination of the visible mucous membranes they showed a distinct yellowish-brown colour. The sheep was very weak and died 12 hours later. Reddish black urine was passed on the last day of life.

Post-Mortem Report (Sheep 15755).

Blood, reddish brown, watery and uncoagulated.

Subcutis, fat, lemon yellow in colour.

Lungs, marked hyperaemia with injection of the vessels of the bronchi.

Heart, nothing unusual.

Liver, light greenish brown in colour on section. Centres of the lobules show marked greenish brown pigmentation.

Spleen, pulp greatly swollen, reddish black in colour, and soft in consistence.

Kidneys, cortex light brown in colour, malpighian bodies distinct.

Large intestine, dark brown, dry faeces.

Bladder, empty.

From this brief post-mortem report and description of the symptoms during life it will be seen that this case was very similar indeed to the cases of so-called bacterial icterus described by the writer (1928).

In both conditions the symptoms and lesions are due to a sudden and marked haemolysis.

Conclusions.—*Corynebacterium ovis* (B. of Preisz Nocard) may cause symptoms due to haemolysis when inoculated intravenously into sheep. When inoculated subcutaneously lesions associated with progressive anaemia may develop.

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15th Annual Report of the Director of Veterinary Services,
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The Preparation of Contagious Abortion Vaccine on Solid Media.

By C. P. NESER, B.A., D.Sc., M.R.C.V.S., Research Officer,
Onderstepoort, Pretoria.

Contagious abortion is very widely spread in the Union of South Africa, and the number of calves lost through it must run into many thousands annually. It is unfortunate that ideal measures of control cannot be applied under the ranching and semi-ranching conditions of South Africa.

For the above reasons it became imperative to supply cattle owners with some product whereby calf loss would be reduced to a minimum. After some experiments that need not be cited here, it was decided to prepare a vaccine on solid media, and to issue this to farmers. It is not the object of this short note to discuss the merits of this (or any other) vaccine, but merely to describe a simple and effective method of preparing it. To discuss the early difficulties encountered would serve no useful purpose, and only an account of the present method of preparing contagious abortion vaccine is therefore given.

Medium.—The medium used is that described by Stafseth (1920), which consists of liver broth agar, containing 1 per cent. glucose or starch, and which has a p.H. of 6.5. No gentian violet is added. This medium is sterilized at a temperature not exceeding 115° C. for half an hour. If a higher temperature is used the sugar or starch becomes slightly charred, and then the *Bacillus abortus* Bang will grow but poorly if at all.

The flasks are conical in shape, 10 inches in diameter at the base, about 8 inches high and have a neck 1½ inch long and 2 inches in diameter. This neck allows of the seeding and assembling tubes (to be referred to later) being very securely fixed in position by a tight cotton wool plug as is shown in the photo.

The seeding tube (A in the photo) is about 1 cm. in diameter, and about 4 inches long. The one end is bordered so that a cotton wool plug can be inserted easily.

The assembling tube (B in the photo) is bent as shown in the photo. This tube is rounded off at both ends, and in addition that part of it just above the medium (b) is somewhat constricted.

When these two tubes have been securely plugged in position, the cotton wool plug is held in position by thick brown paper (as is clearly shown in the photo). Plugs of cotton wool are now inserted in the ends of the seeding and assembling tubes respectively, and these are in turn covered by thick brown paper. The flask is now sterilized at a fairly low temperature in the autoclave.

The medium is heated to about 60° C. and 300 c.c. of it poured into each flask. After the plugs containing the seeding and assembling tubes have been inserted and properly secured, the flasks are transferred to the autoclave for half an hour at the specified temperature. The flasks, ready for seeding, are then placed in an incubator, at 37° C. for at least two days, the object being (1) to ensure that only sterile flasks are seeded, and (2) to dry the medium somewhat.

Seeding is done with a selected strain of Bang's bacillus which has been grown on agar slants. Under aseptic precautions about 10 c.c. normal saline is added to each slant which appears to be a pure culture of Bang's bacillus. The tubes are now rotated between the fingers and a thick suspension of the organisms is obtained. By means of an ordinary glass pipette about 2 c.c. of this suspension is added to each flask through the seeding tube (A), care being taken to use the flame freely but not to overheat and destroy the organisms. After seeding, the cotton wool plugs are replaced and secured as described. From any one slant not more than four flasks are seeded. In order to ensure a good growth the whole of the agar surface must be moistened with the suspension of organisms.

Incubation takes place in a specially constructed room kept at 37° C. by means of an electrical device. No gas-producing organisms should be in this incubator with the contagious abortion vaccine, as growth may be retarded if not completely inhibited. The flasks are incubated from five to seven days, and a massive growth is as a rule obtained. The flasks are inspected daily, and obviously contaminated ones are at once removed.

After suitable incubation the flasks are brought into the main laboratory, and from 100 c.c. to 150 c.c. carbol saline is added [through the seeding tube (A)] to each, according to the growth of organisms on the nutrient medium. The flasks are then shaken in such a way that a slab of agar breaks off: Thereafter the shaking is gentle, the broken piece of agar serving the purpose of detaching the organisms from the surface of the medium. Immediately after the growth has been washed off, a smear is made, a long thin pipette being introduced through the seeding tube to remove a small amount of fluid for this purpose. The flasks and smears are given corresponding numbers. Only those flasks apparently free from contamination according to the smear examination are finally used to prepare the vaccine, the infected flasks being immediately removed and sterilized.

Assembling should now be proceeded with at once. For this purpose we use (1) a vacuum pump or ordinary water siphon (not shown in the photo); (2) a wire gauze filter which is suitably arranged in a flask. One of the standard flasks is used. It is fitted with a rubber cork through which pass an assembling tube, a suction tube (S) and a receiving tube (R), the last two projecting about 4 inches below the cork. By means of a blunt rod the wire gauze is pushed into the flask and opened in the manner indicated in the photo (G); (3) a canvas filter (c) which is prepared in much the same way as the gauze filter with this difference that the canvas bag is tied tightly round the receiving tube which is bordered to prevent slipping; (4) a large receiving flask (x) whose neck is tightly plugged with cotton wool through which pass a receiving tube (R) and a bottling tube Y.

Suitable lengths of thick rubber tubing are secured on the receiving tubes and the bottling tube. The assembling and suction tubes are plugged with cotton wool and secured with brown paper and string as in the case of the culture flasks. An ordinary glass stopper is inserted into the free end of every piece of rubber tubing leading from the assembling tubes and an ordinary bottling piece attached to the free end of the piece of rubber tubing leading from the bottling tube. These are now covered with brown paper, which is secured in position by string. The whole of the assembling apparatus is sterilized in the autoclave at a low temperature (high temperatures cause the rubber tubes to perish rapidly).

While the smears are being stained the tubes of the assembling apparatus are connected as is shown in the photo. As a rule, however, the tube (Z) from the water siphon is first attached to the suction tube S of the gauze filter, and the rubber tubing between the gauze and canvas filter is clamped as is shown in the photo at I.

Proceed as follows: Turn on the water siphon or vacuum pump, and clamp the rubber tubing attached to the receiving tube as is shown in the photo at the point 2. The brown paper and cotton plug are now removed from the assembling tube (B) of the first flask, and the glass tube is kept sterile by freely using the flame without overheating. The free end of the rubber tubing leading from the receiving tube of the gauze filter is slipped over the assembling tube of the first flask, and the clamp at 2 is released. By tilting the culture flask all the fluid with the suspended organisms is sucked into the gauze filter, where all the large pieces of agar are arrested. The reason for constricting the one end of the assembling tube is apparent, because very large pieces of agar cannot enter, and consequently obstruction in any other part of the tubing is avoided. After all the fluid has been drawn from the first culture flask the clamp at (2) is again tightened and the rubber tube is slipped over the assembling tube of the second flask, etc.

When the flask containing the gauze filter is nearly full the siphon tube is attached to the suction tube (S) of the flask containing the canvas filter, and the clamp (1) is released. The rubber tube leading from the assembling tube (B) of the canvas filter is now clamped and the fluid in the gauze filter is sucked through the canvas filter. In a similar manner, but without using the siphon, the suspension is finally passed into the large flask X. After the suspension from all the flasks has been collected in the large flask the rubber tubing is slipped from the assembling tube (B) of the canvas filter and a concentrated alcoholic solution of gentian violet is added so that the ultimate dilution of this drug is 1 in 5,000. Through this same tube 40 per cent. glycerine saline is then allowed to run into the flask X, until the volume is exactly doubled. The rubber tubing is then securely clamped near the receiving tube of the flask X. After a thorough shaking, bottling can be proceeded with in the ordinary way.

DISCUSSION.

The technique described is simple, and the chances of the vaccine becoming contaminated are reduced to a minimum. The fact that Bang's bacillus in suspension lives a very much longer time than was previously thought to be the case (Neser, 1926), enables us to make very large batches at a time with the result that the cost of production is reduced considerably. Moreover, it takes at most a week to prepare a batch, and this is a decided advantage over the method used by Stockman, with his now famous potato medium (referred to in standard books).

At one time Stockman did use ordinary agar and prepared a vaccine on this medium. This method, however, for various reasons, was later abandoned in favour of a method in which a fluid media was used. In order to obtain a heavy growth such flasks have to be incubated for several weeks, and in addition it is uncertain whether the *Bacillus abortus* Bang will then live as long as when grown on solid fairly dry agar. Experience has shown that the batches of vaccine grown on fairly dry medium as described, are always more satisfactory in that abscesses follow less frequently after inoculation, and the bacilli do not die soon.

It may be stated that small animals (guinea-pigs) are always injected with a dose of 2 c.c. subcutaneously and intraperitoneally before a batch is issued. If peritonitis or an abscess follows this test, the entire batch of vaccine is discarded, but if the guinea-pigs are healthy two days later, the vaccine is issued. Bottling usually takes place after this test.

The vaccine is put up in bottles in amounts of 100 c.c., 50 c.c., 20 c.c., and 10 c.c. : the dose is 10 c.c. subcutaneously. This small dose is a great advantage, and probably accounts to some extent for the fact that users very rarely report abscesses. The bottled vaccine is kept in cold storage. If a batch is more than a month old, a small bottle is opened, and cultures made, in order to make quite certain that live vaccine is issued.

Before issuing vaccine we warn owners about the danger in connection with its use, and whenever possible we first of all make quite certain that it is contagious abortion by applying the agglutination test. Certain users assure us that the vaccine, while giving a good immunity, never brings about abortion, even if injected into pregnant animals. While this statement cannot be acceptable as literally correct, there is no doubt that the vaccine strain is very much attenuated, because we never now find any lesions in the test guinea-pigs. The agglutination following the use of the vaccine is of a very high order, and in no way less than that following the use of a virulent strain that always produces lesions in guinea-pigs.

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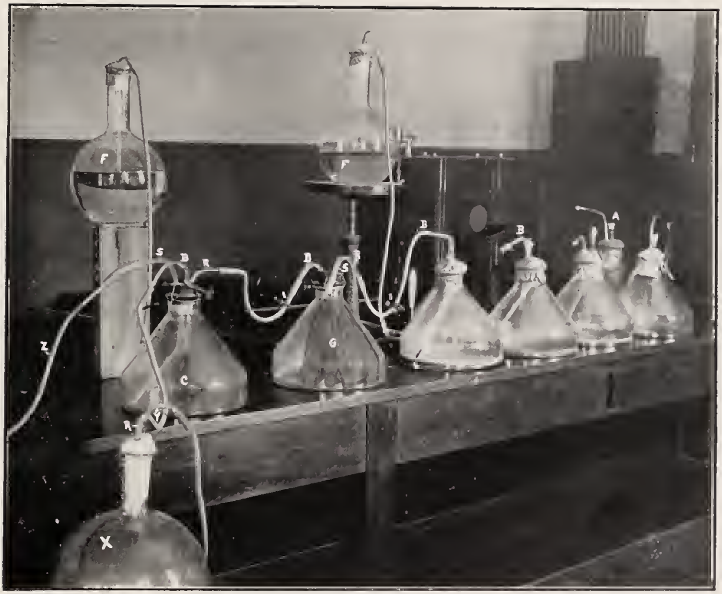

Apparatus for preparation of contagious abortion vaccine on solid media.

A. Seeding tube. B. Assembling tube. S. Suction tube. R. Receiving tube. G. Wire gauze. C. Canvas filter. X. Large receiving flask. Y. Bottling tube. Z. Tube from water siphon.

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Studies on Anthrax Immunity.

By J. I. QUIN, B.V.Sc., Veterinary Research Officer, Onderstepoort Laboratories.

Thesis accepted for the degree of Doctor of Veterinary Science by the University of South Africa.

INTRODUCTION.

Although in the past the term Anthrax was used rather loosely for different diseases, there seems to be little doubt that the condition as we know it to-day has been in existence since the earliest times. In fact, several of the ancient authors (Homer, Ovid and Pliny) wrote about a disease most probably identical with anthrax. That anthrax is a disease of primary significance may be gauged from the fact that it has meant the death of millions of animals as well as many thousands of human beings. The aetiology of the disease was completey unknown until Robert Koch in 1876 proved the significance of the rod-shaped organisms in the blood, subsequently termed the Bacillus anthracis. Shortly afterwards Louis Pasteur succeeded in producing a vaccine against the malady. Since the discovery of the causal organism, the disease has received the attention of hundreds of investigators from all parts of the world, and an enormous amount of literature has been published. Even at the present moment research work in connection with anthrax is being conducted on a large scale, for, although we possess valuable weapons for combating the disease, some cardinal points are as yet unexplained. As examples, one may mention the routes of infection in the different classes of animals, the pathogenesis of the disease and the biology of the organism in nature outside the animal body.

With reference to the position of anthrax in South Africa up to the end of 1924, Viljoen, Curson and Fourie have recently published a detailed report in which the occurrence, diagnosis and preventive measures are fully discussed. Previous to that Kehoe and others have written on the disease in South Africa.

In this report is embodied the greater part of the experimental work carried out, and the observations made by the writer during the years 1925 to 1928, while working in the Anthrax Section of the Veterinary Research Laboratory, Onderstepoort.

It is a well-known fact that apart from the actual destruction of all anthrax-infected material, the active immunization of animals against the disease is by far the most effective means we possess of eradicating it. For this purpose three main types of vaccine are used, more or less exclusively, all over the world:—

(*a*) *Broth cultures* containing the living but much weakened or attenuated vegetative forms of the Bacillus anthracis. This vaccine was first introduced by Louis Pasteur, and is still used extensively in certain countries. It is generally prepared as a double vaccine, the first inoculation being made with a weaker vaccine which produces the ground immunity, while the second, which is stronger, is considered to enhance the immunity.

(*b*) *Anthrax Spore Vaccine.*—This vaccine, which is now favoured in many countries, is of comparatively recent introduction, although based on the same principle as that employed for the Pasteur vaccine, i.e. the utilisation of attenuated living organisms. It differs, however, in that the more resistant spores of the attenuated vegetative forms are used rather than the bacilli themselves. Vegetation of the spores in the vaccine is prevented by suspending these in various preservatives, e.g. glycerinised saline. By this procedure the keeping qualities as well as the uniformity of the vaccine are markedly improved, while the immunity conferred is in no way inferior to that produced by the Pasteur vaccine.

(*c*) *Sero-vaccination as advocated by Sobernheim.*—This method, which is extensively used in certain European countries as well as in South America, consists of a simultaneous injection of anthrax immune serum, together with attenuated anthrax cultures. The immune serum produces an immediate passive immunity, while the effect of the living but attenuated organisms is considered to be a rapid replacement of the passive by a solid active immunity against anthrax.

Further, it may be briefly mentioned that a fourth method of preventive and curative inoculation exists, viz. by means of hyperimmune anthrax sera alone, the use of which, however, is markedly limited in comparison with the above-mentioned vaccines, and the immunity conferred of a purely passive and transitory nature.

Although the three types of vaccine mentioned are extensively used, and generally with good results, several investigators, such as Bail, have from time to time been engaged in an endeavour to demonstrate the presence, and value as actively immunizing agents, of non-living metabolic products of the anthrax organism, elaborated either artificially in vitro or in the animal body. It may be stated that up to the present the results of these researches have been most conflicting; e.g. some authors claim to have demonstrated definitely toxic action of these products, while others have failed to do so. Similarly in some cases active immunity seemed to have been provoked, while in other cases the results were clearly disappointing. Should it, however, be clearly demonstrated that the anthrax bacillus is capable of elaborating a potent immunizing substance comparable to the so-called "aggressin" of black-quarter, it would indeed be of inestimable value in fighting the disease.

However efficient our present anthrax vaccines may be, it should always be borne in mind that the wholesale inoculation with live organisms, although attenuated, is far from ideal, since they are directly derived from the dangerously virulent organism, the biology of which is not completely known. Further, it may be noted that some difficulty is experienced in immunizing certain classes of animals, e.g. goats, which are very susceptible to anthrax and may die as the result of vaccination, while horses are peculiarly susceptible to the development of subcutaneous swellings following on vaccination.

During the course of the last few years, what is described as " true Anthrax aggressin " has appeared on the market, and complete safety and strong immunizing properties are claimed for it.

The object of the experimental work carried out by the author was as follows:—

(*a*) To attempt the production of a non-living yet efficient immunizing substance against anthrax.

(*b*) To compare the relative values of different methods of immunization with anthrax spore vaccine. The latter experiments were conducted mainly with the view of ascertaining the value of cuti-vaccination, as advocated by Besredka and others, in comparison with the methods usually employed in South Africa.

The work is presented in several sections and discussed under the following titles. Tables and protocols of experiments are included in the text in preference to placing them together in a special appendix.

1. Species susceptibility and immunity.
   The selection of experimental animals.
2. Anthrax cultivation experiments.
   (*a*) Attenuation of virulent strains.
   (*b*) Experiments with disintegrated cultures.
   (*c*) Experiments with washed cultures.
3. Production of immunizing or toxic substances.
   (*a*) Artificial in vitro experiments.
   (*b*) Natural, in the animal body.
4. Comparative safety and immunity experiments with anthrax spore vaccine.
5. Summary and general discussion.
6. Conclusions.
7. Bibliography.

1. SPECIES SUSCEPTIBILITY AND IMMUNITY.

THE SELECTION OF EXPERIMENTAL ANIMALS.

Numerous investigators, including Sobernheim, Baumgarten, Löffler, Metchnikoff, and K. Müller, have pointed out the fact that different species of animals exhibit marked variations in the degree of susceptibility to anthrax both under natural and artificial conditions. Even in the same class of animal these differences are frequently observed, e.g. whereas poultry show marked resistance, Robertson found the ostrich peculiarly susceptible to the disease. According to K Müller, the different species of rats exhibit marked variations in susceptibility to anthrax. Carnivorous animals are as a general rule far more resistant than herbivora. Furthermore, differences may be demonstrated in one and the same species of animal when kept under different conditions. It has frequently been observed that, although such animals as cattle and horses are markedly susceptible, and may die in large numbers from anthrax naturally contracted, it is with great difficulty that a fatal artificial infection can be brought about either by enternal or parenteral administration of virulent anthrax material. As a result of our incomplete knowledge as to the natural routes of infection, this peculiar phenomenon is still a mystery. On the other hand may be mentioned the ease with which sheep, goats and most laboratory animals can be fatally infected.

With reference to immunity, as has been mentioned before, certain species of animals exhibit a marked inherent resistance to the disease, while most of the susceptible species can be successfully immunized by the use of reliable vaccines.

d'Herelle expressed the opinion that the study of immunity in artificially produced infectious diseases does not permit one to assume that the same conditions will prevail in natural circumstances. This point is of great significance and should not be lost sight of.

Among the various susceptible species, however, notable exceptions are encountered. Before any immunity work on anthrax is carried out, it is essential that these points be clearly realized, and it is for this reason that this aspect is particularly stressed. Thus it may happen that experiments carried out on certain animal species which are markedly susceptible but difficult to immunize may result in totally wrong conclusions being drawn. Many of the conflicting results obtained by different workers may probably be ascribed to the different types of animals used.

The ideal species of animal to be used in immunity experiments would obviously be one showing a practically constant and well marked susceptibility to artificial infection combined with the quality of developing a solid immunity after vaccination. It is intended to discuss briefly the more common domesticated animals from this point of view.

(*a*) *The Common Laboratory Animals.*—Of these may be mentioned the white rat, white mouse, the guineapig and the rabbit. Among these animals, it is exceedingly rare for a spontaneous outbreak of anthrax to occur, although all are easily infected artificially except the white rat, which exhibits a strong natural immunity. White mice may be killed even by very weak vaccines. Should they survive vaccination, the degree of immunity, if any be developed, is, however, negligible. Similarly guineapigs, which are extremely susceptble even to weak strains, are particularly difficult, and perhaps impossible, to immunize, and hence great care should be exercised in drawing conclusions from any immunity experiments carried out on these animals. Rabbits, which are markedly susceptible to virulent but less so to vaccine strains, may develop a certain degree of immunity which, however, is weak and always unreliable. Although these points are vaguely known, they are not fully appreciated, with the result that erroneous conclusions may be arrived at.

(*b*) *Goats.*—The animals here referred to comprise the domesticated species indigenous to South Africa. These animals exhibit a well-marked susceptibility both to natural and artificial infection with anthrax. Deaths may occur at times even from the use of weak vaccines. The immunity developed, although as a rule fairly strong, is inclined to be inconsistent.

(*c*) *Sheep.*—Although under natural conditions outbreaks of anthrax appear to be less frequent than among cattle, merino sheep exhibit a well-marked and practically uniform susceptibility to artificial infection with virulent material. At the same time the losses, even from strong vaccine, are very small, while a solid and reliable immunity is developed.

(*d*) *Cattle.*—Under natural conditions cattle are perhaps more subject to anthrax than any other species of animal, although great difficulty may be experienced in attempting to kill them by means of artificial infection. That a strong immunity has been developed after vaccination can only be ascertained by the effective stoppage of an active outbreak.

(*e*) *Horses.*—These animals show a peculiar susceptibility to anthrax under natural conditions. Definite symptoms, generally of a subacute or even chronic nature, may be noted, the chief of which are the large oedematous swellings in the subcutis of certain regions of the body.

The results of artificial infection, however, are most inconsistent. Thus it frequently happens that animals withstand even large injections of virulent material. On the other hand alarming swellings may follow the injection of small doses of weak vaccine. This phenomenon is as yet unexplained.

To substantiate the above views frequent examples will be mentioned in the text.

It will thus be clear, firstly, that although the small laboratory animals are markedly susceptible to artificial infection, the subsequent immunity developed in surviving cases as tested with more virulent material is unreliable; secondly, susceptible cattle and horses withstand virulent artificial infection in a large percentage of cases. It thus becomes exceedingly difficult to ascertain the degree of immunity developed after vaccination as subsequently tested with fully virulent material.

From the numerous immunity experiments conducted, it was clearly evident that only in very few species could reliable information be obtained as to the actual degree of immunity developed. For this purpose merino sheep were found to yield by far the most constant and reliable results, and consequently practically all the immunity work was carried out on this species. To a lesser extent goats were also used.

On account of the wholesale inoculation of farm animals in South Africa against anthrax, attention had to be paid to the source of experimental animals. As anthrax was of uncommon occurrence in the Karroo, and hence practically no animals were vaccinated there against anthrax, most of the susceptible experimental sheep and goats were derived from that region. Such animals generally show a well-marked and practically constant susceptibility to artificial infection.

Horses were used only for the purpose of noting the severity of clinical symptoms following different methods of vaccination.

The small laboratory animals were extensively used mainly to ascertain the degree of virulence of anthrax material, both unattenuated and vaccine strains. Further these animals were used for the purpose of testing the toxicity of different non-living anthrax products.

Except in a few particular cases, cattle were not included in these experiments, on account of the difficulty experienced in killing non-vaccinated controls with virulent material.

2. ANTHRAX CULTIVATION EXPERIMENTS.

(*a*) Attenuation of Virulent Strains.

Toussaint was the first to notice a decrease in the virulence of anthrax organisms in infected blood when exposed for short periods to higher temperatures than the optimal. Through the researches of Pasteur, Chamberland and Roux, a method was devised for the gradual attenuation of the anthrax organism in artificial media. By cultivating the B. anthracis in sterile bouillon at 42° C. for long periods, a progressive decrease in virulence was brought about. It was thus found that such cultures first become avirulent for rabbits, later for guineapigs, and eventually also for white mice. Although avirulent, these organisms were found to confer an immunity against fully virulent cultures. This formed the basis for the famous Pasteur Anthrax Vaccine. Although at the present day numerous other anthrax vaccines are also in use, the principle of attenuation by prolonged incubation at higher temperatures is still maintained in practically every instance. Various other methods have been described for the attenuation of the B. anthracis, although apparently without any distinct advantage over the Pasteur method. Preisz drew attention to the possibility of an unequal attenuation of different anthrax strains, and even of individual organisms.

According to Koch, Gaffky and Löffler, the passage of attenuated vaccine strains through experimental animals does not enhance the virulence of such strains except perhaps where the original virulent organisms were attenuated very rapidly in a short time, i.e. gradual attenuation leads to the production of vaccine strains showing greater constancy. From recent investigations Schilling claims to have shown that anthrax organisms could be attenuated by growing on 5 per cent. sodium chloride agar for six weeks. Rabbits could be successfully and safely immunized, and afterwards withstood virulent anthrax cultures which regularly killed susceptible controls. The same, however, could not be established for guineapigs.

Attenuation of several virulent anthrax strains was attempted in different ways. Pure agar cultures were obtained from six strains of virulent anthrax from different parts of South Africa. All these were definitely proved to be lethal to guineapigs and rabbits. Attenuation was commenced by inoculating broth tubes with the pure strains and consistently incubating these at 42° C. Injections into guineapigs 24 to 48 hours afterwards confirmed the virulence noted in the first instance. After incubating for 25 days, subcultures on to nutrient agar were made every 5 days, incubated at 37° C. for 3-4 days, sealed and kept at room temperature for further use. This was continued until all the strains had been incubated at 42° C. up to a period of 90 days. Following this, so-called " test batches " were prepared from several selected agar tubes representing different periods of attenuation. Young broth cultures with seed material obtained from the agar slants, were sown on to the thin layers of nutrient agar contained in large flat-bottomed Fernbach flasks and incubated at 37° C. Growth and sporulation were noted daily. Generally, after 4 or 5 days, sporulation was practically complete. The growth was then removed by pouring small amounts of sterile normal saline on to the surface, together with a few sterile glass beads. By gentle tilting of the flasks, the growth became dislodged. This was carefully decanted into sterilized flasks and an equal weight of pure glycerine added. This formed the concentrated glycerinised spore emulsion with which all further tests were conducted. Glycerine was found to preserve the spores and prevent vegetation. By this procedure spore emulsions were found to maintain a uniform strength for very long periods. Subsequently the degree of virulence of these experimental vaccines was tested on guineapigs and rabbits by subcutaneous injections of various dilutions made in normal saline. The immunizing properties were afterwards ascertained by vaccination of sheep and goats, but only in those cases where rabbits survived the injection. When rabbits died from the vaccine, it was generally found to cause death in a fair percentage of the sheep and goats. The degree of immunity developed in the sheep and goats were subsequently tested, after 14 to 21 days, by the subcutaneous injection of a virulent glycerinised spore emulsion of known strength. This was prepared in the same way as described for the test batches. By previously conducted experiments the minimal lethal dose, referred to as the M.L.D., for sheep was accurately determined.

The testing of virulent spore emulsion was carried out periodically and the M.L.D. fixed. Diagnosis of anthrax was made by the microscopic examination of blood or spleen smears taken from dead animals. Where these proved to be negative, broth cultures made from the spleen, were injected into white mice and the result noted.

The following experiments will serve to demonstrate the variations in safety and immunizing properties of different test batches:—

EXPERIMENT 1.

Strain A, attenuated 50 days.

1 rabbit injected .1 c.c. glycerine emulsion—dead in 4 days Anthrax.

1 guineapig injected .1 c.c. emulsion—dead in 3 days Anthrax.

1 guineapig injected .01 c.c. emulsion—dead in 3 days Anthrax.

Strain A, attenuated 70 *days.*

1 rabbit injected .1 c.c. emulsion—remained alive.
1 guineapig injected .1 c.c. emulsion—dead in 4 days Anthrax.
1 guineapig injected .01 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected .001 c.c. emulsion—dead in 4 days Anthrax.

As the rabbit remained alive this batch was tested out on sheep and goats.

Animal.	Date Vaccine, 18/8/25.	Result.	Date Virulent Spores, 8/9/25.	Result.
	c.c.		M.L.D.	
Sheep 11324.......	0·02	—	500	Alive.
,, 7349.......	0·02	—	500	,,
,, 11022.......	0·01	—	500	Dead, 15/9/25, Anthrax.
,, 11217.......	0·01	—	250	Alive.
Goat 11850.......	0·02	—	250	,,
,, 11856.......	0·01	Dead, 29/8/25, Anthrax	—	—

One goat is noticed to have died from anthrax after vaccination, while in the immunity test one of the sheep succumbed from anthrax.

Strain A, attenuated 80 *days*—Single Colony.

1 rabbit injected .1 c.c. emulsion—remained alive.
1 guineapig injected .1 c.c. emulsion—dead in 5 days Anthrax.
1 guineapig injected .01 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected .001 c.c. emulsion—remained alive.
This was again tested on sheep and goats as follows:—

Animal.	Date Vaccine, 2/12/25.	Result.	Date Virulent Spores, 18/12/25.	Result.
	c.c.		M.L.D.	
Sheep 9005.......	0·02	—	500	Lived.
,, 8865.......	0·01	—	250	,,
Goat 14031.......	0·02	—	500	,,
,, 14024.......	0·01	—	250	,,

It is seen that this particular batch was both safe for sheep and goats and conferred a good immunity, protecting against as much as 500 M.L.D.

Strain A, attenuated 80 *days.*

From the same tube that the above batch was made, another was prepared, only that a different colony was selected from the agar plate.

1 rabbit injected .1 c.c. emulsion—remained alive.
1 guineapig injected .1 c.c. emulsion—dead in 5 days Anthrax.

1 guineapig injected .01 c.c. emulsion—dead in 5 days Anthrax.
1 guineapig injected .001 c.c. emulsion—dead in 5 days Anthrax.

NOTE:—In this case the .001 c.c. killed, while in the previous batch the guineapig remained alive.

Animal.	Date Vaccine, 2/12/25.	Result.	Date Virulent Spores, 18/12/25.	Result.
	c.c.		M.L.D.	
Sheep. 10647.......	0·02	—	1,000	Alive.
,, 10724.......	0·01	—	500	,,
Goat 14032.......	0·02	Dead, 5/12/25, Anthrax	—	—
,, 14020.......	0·01	Dead, 6/12/25, Anthrax	—	—

In this case the vaccine prepared from a different colony is highly virulent for guineapigs and goats, although it is safe for sheep and confers a strong immunity. This clearly demonstrates the unequal attenuation of different organisms although of the same strain and in the same tube.

EXPERIMENT 2.

Strain B, attenuated 50 days.

1 rabbit injected .1 c.c. emulsion—remained alive.
1 guineapig injected .1 c.c. emulsion—remained alive.
1 guineapig injected .01 c.c. emulsion—dead in 7 days Anthrax.
1 guineapig injected .001 c.c. emulsion—remained alive.

This is obviously a very weak vaccine. Tested out on sheep and goats, it gave the following peculiar results:—

Animal.	Date Vaccine, 26/10/25.	Result.	Date Virulent Spores, 9/11/25.	Result.
	c.c.		M.L.D.	
Sheep 12249.......	0·02	—	500	Dead, 14/11/25, Anthrax.
,, 9524.......	0·01	—	250	Dead, 12/11/25, Anthrax.
Goat 11859.......	0·02	—	250	Alive.
,, 11854.......	0·01	—	100	,,

In this case, the vaccine, besides being safe for sheep and goats, immunized the two goats, while the two sheep readily succumbed to the immunity test.

Strain B, attenuated 75 days.

1 rabbit injected .1 c.c. emulsion—dead in 7 days Anthrax.
1 guineapig injected .1 c.c. emulsion—dead in 3 days Anthrax.

1 guineapig injected .001 c.c. emulsion—dead in 4 days Anthrax.
1 guineapig injected .01 c.c. emulsion—dead in 3 days Anthrax.

This batch is markedly virulent although the strain was attenuated for 75 days. It would appear that the colony from which it was prepared was formed from an organism in which no attenuation had taken place. It was not tested on sheep since it killed the rabbit.

In this way no less than 65 test batches were prepared from different attenuations, selecting different colonies of the six strains. These were all tested out on rabbits and guineapigs and when promising, also on sheep and goats. Of these not more than four could be considered of any value as reliable vaccine strains. In numerous cases either the vaccine killed or in the surviving animals immunity was absent or weak.

It was clearly evident that the production of a reliable vaccine strain, i.e. one in which safety and immunity were combined, depended on at least two factors, viz.: (*a*) the strain itself, (*b*) individual organisms comprising the strain. Some strains proved to be altogether useless, while in other strains certain colonies yielded excellent test batches.

In some cases it was found necessary to attenuate up to 90 days before the requisite attenuation was reached, while with other strains attenuation for 45 days proved sufficient. It seemed definite that no fixed period could be prescribed for attenuation, and that constant testing out on animals was the only reliable way of obtaining a suitable vaccine strain.

It was then decided to attempt the attenuation of a virulent anthrax strain by prolonged cultivation in a number of different liquid media. This strain, which was known to be markedly virulent in the original state, had been attenuated previously by other means, yielding an excellent vaccine strain. The special media were prepared in the same way as ordinary nutrient broth, except that various other materials were substituted for the minced beef. The sterile medium contained in a litre flask was inoculated with 5 c.c. of a 24-hour broth culture of the virulent strain, and incubated for 30 days at a temperature of 37° C. Guineapigs and rabbits were then inoculated subcutaneously with varying amounts of the culture and the results noted.

EXPERIMENT 3.

Controls. Virulent Anthrax 24 hour broth culture.

1 rabbit injected .5 c.c.—dead in 3 days Anthrax.
1 guineapig injected .5 c.c.—dead in 2 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

Controls. Virulent Anthrax 30 day broth culture.

1 rabbit injected .5 c.c.—dead in 4 days Anthrax.
1 guineapig injected .5 c.c.—dead in 3 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

A. Virulent Anthrax in Pea broth for 30 days.

(Ordinary dry peas were used.)

1 rabbit injected .5 c.c.—dead in 4 days Anthrax.
1 guineapig injected .5 c.c.—dead in 2 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

B. Virulent Anthrax in Bean broth for 30 days.

(Ordinary dry beans were used.)

1 rabbit injected .5 c.c.—dead in 5 days Anthrax.
1 guineapig injected .5 c.c.—dead in 2 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

C. Virulent Anthrax in ordinary broth + whole egg for 30 days.

1 rabbit injected .5 c.c.—dead in 5 days Anthrax.
1 guineapig injected .5 c.c.—dead in 2 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

D. Virulent Anthrax in Chicken muscle broth for 30 days.

1 rabbit injected .5 c.c.—dead in 5 days Anthrax.
1 guineapig injected .5 c.c.—dead in 2 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

E. Virulent Anthrax in Dog muscle broth for 30 days.

1 rabbit injected .5 c.c.—dead in 3 days Anthrax.
1 guineapig injected .5 c.c.—dead in 3 days Anthrax.
1 guineapig injected .25 c.c.—dead in 4 days Anthrax.

F. Virulent Anthrax in Potato broth for 30 days.

1 rabbit injected .5 c.c.—dead in 4 days Anthrax.
1 guineapig injected .5 c.c.—dead in 4 days Anthrax.
1 guineapig injected .25 c.c.—dead in 2 days Anthrax.

G. Virulent Anthrax in ordinary broth + 10 per cent. Anthrax hyperimmune Donkey serum for 30 days.

1 rabbit injected .5 c.c.—dead in 5 days Anthrax.
1 guineapig injected .5 c.c.—dead in 2 days Anthrax.
1 guineapig injected .25 c.c.—dead in 3 days Anthrax.

It is thus seen that, out of a total of 27 animals injected with the same amounts of different anthrax cultures, not a single one survived, showing that practically no attenuation had taken place in any of the flasks. The incubation was stopped at 30 days, since after this period attenuation might have been expected even in the plain broth control cultures. From these experiments it was evident that by cultivating virulent anthrax organisms in these various media, attenuation was brought about no sooner than in ordinary broth.

Attempted attenuation by freezing and thawing.

As a third method of attenuating virulent anthrax cultures it was decided to attempt this by repeatedly freezing and thawing of the cultures. For this purpose a small apparatus was constructed, consisting of two tubes closed at one end made out of galvanized iron and suspended one within the other. A small inlet in the outer tube was connected with a cylinder containing carbon dioxide under high pressure. A saline emulsion consisting of a 12 hour agar growth of virulent anthrax organisms was placed in the inner tube. Opening of the cyclinder caused the rapid escape of Co_2 from between the two tubes. This resulted in almost immediate freezing of the emulsion. After being frozen for 10 minutes the inner tube was removed and quickly steeped into water at 90° C. This caused rapid thawing inside the tube. As soon as liquefaction was complete the freezing was repeated. Subcultures on nutrient agar were made after every alternate freezing and incubated at 37° C. This freezing and thawing was continued 24 times in succession. After incubating the agar subcultures for 24 hours, approximately equal amounts of growth (one platinum loopful) were removed and suspended in definite amounts of saline. This was subsequently injected into guineapigs and rabbits with the following results:—

EXPERIMENT 4.

Virulent Control.—24 hour old virulent agar culture, one loopful suspended in 10 c.c. saline.

1 rabbit injected 1 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected 1 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected .1 c.c. emulsion—dead in 3 days Anthrax.

Frozen 1.

One loopful 24 hour agar culture made from material frozen once and suspended in 10 c.c. saline.

1 rabbit injected 1 c.c. emulsion—dead in 7 days Anthrax.
1 guineapig injected 1 c.c. emulsion—dead in 2 days Anthrax.
1 guineapig injected .1 c.c. emulsion—dead in 5 days Anthrax.

Frozen 21.

This was made from material frozen 21 times.

1 rabbit injected 1 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected 1 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected .1 c.c. emulsion—dead in 3 days Anthrax.

Frozen 24.

As the frozen 21 showed no signs of attenuation, the frozen 23 culture was taken and frozen for two days on end and termed frozen 24. Saline emulsions were made as above and guineapigs and rabbits injected only with much smaller amounts.

1 rabbit injected .001 c.c. emulsion—dead in 3 days Anthrax.
1 guineapig injected .001 c.c. emulsion—dead in 2 days Anthrax.
1 guineapig injected .0001 c.c. emulsion—dead in 5 days Anthrax.
1 guineapig injected .00001 c.c. emulsion—dead in 3 days Anthrax.

From the above experiment it was clear that the young 12 hour old vegetative forms of virulent anthrax could withstand with impunity persistent rapid alternate freezing and thawing. Microscopic examination of smears made from the frozen material revealed not the slightest signs of disintegration, while the animal tests proved the absence of any attenuation having taken place.

Of the different methods of attenuation attempted, it was decided that the Pasteur method, although tedious and frequently disappointing, was the only one in which attenuation definitely took place.

(*b*) EXPERIMENTS WITH DISINTEGRATED ANTHRAX CULTURES.

The object of this series of experiments was to devise ways and means for the actual disintegration of anthrax organisms and setting free of the protoplasm. Once this had been achieved the sterile germ-free product was to be tested out on small laboratory animals in order to ascertain the presence or absence of endocellular toxins, and further to note the degree of immunity developed, if any, in sheep and goats. For these experiments the growth from young 12-24 hour old agar slopes was used, the seed material consisting of a highly virulent anthrax strain. The young growth consisting mostly of vegetative forms, was carefully removed from the agar surface by means of a platinum needle and suspended in normal saline. In this way a thick saline emulsion was formed.

As mentioned before, the alternate freezing and thawing of such emulsions did not cause disintegration and hence the method was abandoned.

Several other methods employed gave completely satisfactory results.

EXPERIMENT 5.

Disintegration by continued shaking.

A saline emulsion prepared as described above was placed in a strong flask together with a number of sterile glass beads and securely corked. It was then shaken continuously in a shaking apparatus for four days.

Microscopic examination revealed the presence of a large percentage of disintegrated bacilli in the form of debris. Subcultivation, however, resulted in an abundant growth. The emulsion was, therefore, diluted and carefully passed through a Seitz bacterial filter. The filtrate was completely sterile as shown by subcultivation and smears. The material was then injected into small animals as follows:—

1 guineapig 2 c.c. filtrate—remained alive.
1 guineapig 2 c.c. filtrate—remained alive.
1 white mouse 1 c.c. filtrate—remained alive.

Similarly a young broth culture shaken up in the same way, filtered and injected into small animals produced no effect.

The addition of 1 in 1,000 solution of anilin gentian violet to the saline emulsion was also found to cause rapid disintegration of the bacilli and sterilization of the emulsion.

Two guineapigs injected with 1 c.c. each of the emulsion—both remained alive. Similarly two white mice remained alive. This material was also tested out on two sheep:

Animal.	Date Emulsion, 16/9/26.	Result.	Date Virulent Spores, 29/9/26.	Result.
	c.c.		M.L.D.	
Sheep 7716........	5	—	100	Dead, 3/10/26. Anthrax.
,, 8722........	3	—	100	Dead, 2/10/26, Anthrax.

Disintegration of virulent material was also carried out by grinding it up in a mortar. after adding about half the volume of clean sterile sand. In this way practically all the bacilli were disintegrated. After passing the diluted saline emulsion through a bacterial filter, and confirming sterility, the filtrate was again tested out on animals. Guineapigs and white mice injected with amounts up to 3 c.c. all remained alive. Sheep were injected as follows:—

Animal.	Date Filtrate, 21/10/26.	Result.	Date Virulent Spores, 1/11/26.	Result.
	c.c. filtrate.		M.L.D.	
Sheep 14677.......	5	—	100	Dead, 5/11/26, Anthrax.
,, 14680.......	10	—	100	Remained alive.

As one sheep remained alive the experiment was repeated on two more sheep, injecting the same dose of filtrate and the same amount of virulent material, with the result that both died.

From these experiments it was clear that artificially cultivated young bacilli may be regarded as containing no endocellular toxic substance sufficient to kill a mouse. Similarly it was devoid of any substance capable of conferring immunity on sheep.

(*c*) Experiments with Washed Cultures.

The object of these experiments was to determine the virulence and also the immunizing powers of anthrax organisms after complete removal of all traces of their extracellular products of metabolism. This was carried out by the repeated washing in normal saline and centrifugalizing of the organisms. It has been shown that certain bacteria, e.g. the Bacillus chauveaui of black-quarter, when completely separated from all extracellular products, both the vegetative forms and the spores are incapable of setting up infection and causing death.

Experiments on anthrax were carried out as follows:—

EXPERIMENT 6—SPORES ONLY.

For this the glycerinised spore emulsion of a fairly strong and very reliable vaccine batch was used. Microscopic examination of smears revealed the presence of spores only. A small amount of glycerinised emulsion was diluted in saline and thoroughly centrifuged. The supernatant fluid was siphoned off and the precipitate consisting of spores again shaken up with saline and the centrifuging repeated. This was done six times until probably all traces of extracellular substances had been removed. Various saline dilutions of the spores were then made up and injected into small animals as follows:—

1 guineapig—1 c.c. washed spores	All animals died within 4 days from Anthrax.
1 guineapig—1 c.c. of .1 dilution spores	
1 guineapig—1 c.c. of .01 dilution spores	
1 guineapig—1 c.c. of .001 dilution spores	

Four white mice injected with the same amounts also died within four days. The material was then tested on sheep as follows:—

Animal.	Date Washed Vaccine Spores, 25/11/26.	Result.	Date Virulent Spores, 6/12/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 15623.......	1	—	500	Remained alive.
,, 14338.......	·001	—	250	,,

This experiment therefore clearly demonstrated that the Bacillus anthracis (in the spore stage) unlike the Bacillus chauveaui is capable of causing death as also of immunizing sheep without the presence of any extracellular products i.e. toxins or aggressins.

EXPERIMENT 7—VEGETATIVE FORMS ONLY.

The spore emulsion from the same vaccine batch was inoculated into broth tubes and incubated for 24 hours. Microscopic examination revealed numerous bacilli but no spores. The broth culture was then centrifugalized and the precipitate of bacilli repeatedly washed as in the previous experiment. Various dilutions of the bacilli were again injected into small animals, guineapigs and white mice. These all died from anthrax following injections of dilutions up to .01 of the original suspension of bacilli. Sheep were also tested as follows:—

Animal.	Date Washed Vaccine Spores, 25/11/26.	Result.	Date Virulent Spores, 6/12/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 15621.......	1	—	500	Remained alive.
,, 15592.......	0·0001	—	250	Dead, 9/12/26, Anthrax.

The amount used on the second sheep was obviously too small and on retesting another animal with a larger amount it withstood the immunity test.

From this experiment, as from the previous one, it was clear that the washed bacilli were capable of killing small animals and of immunizing sheep.

3. PRODUCTION OF IMMUNIZING OR TOXIC SUBSTANCES.

(*a*) Artificial in vitro Experiments.

Numerous investigations have been conducted with the object of ascertaining the actual cause of death following a fatal anthrax infection. The view is frequently held that the tremendous accumulation of anthrax bacilli in the general blood circulation towards the end of an infection is in itself sufficient to cause death, i.e. by purely mechanical means through a blockage of numerous capillaries supplying vital organs. According to this view death could result from internal asphyxia. Other authors again maintain that it may be due to pulmonary embolism. Although apparently of great significance, this mechanical factor alone does not explain the cause of death in every instance, since it may happen that death takes place without the presence of many organisms in the circulation. This is especially noticeable in equines where only very few organisms may be found in the blood after death. The possibility of toxic substances elaborated by the B. anthracis has, therefore, to be considered. From this point the results of the investigations of the various authors are most conflicting.

According to Pasteur and Joubert, Levy and Beckman, Sanarelli, Hruska and others, no toxins are elaborated by the B. anthracis. Conradi claims to have shown that neither endo- nor extracellular toxins are formed by the B. anthracis whether cultivated in vitro or in the animal body. Against this many workers claim to have demonstrated definitely toxic substances formed by the anthrax organism. Hoffa, Hankin and Westbrook, Martin, Brieger and Fraenkel, Marmier and more recently Markoff, Bierbaum and Boehnke, all maintain that toxins, capable of causing definite symptoms and even death, are formed by anthrax organisms. Further, the immunizing property of these metabolic products of the B. anthracis has formed the subject for close investigation. Here again the evidence brought forward is very conflicting, some authors claiming reliable immunizing properties for certain germ-free anthrax products, while others report completely negative results.

Bail was the first to apply his conception of aggressin immunity to anthrax. By the use of sterile material collected from the oedematous infiltrations of animals dead from anthrax, he was able to cause a strong immunity in sheep, rabbits and guineapigs. That this was not of the nature of a passive immunity was shown by the fact that it only developed 8 to 10 days after vaccination of the animals. Markoff also found shaked extracts prepared from anthrax cultures protecting rabbits against a subsequent virulent infection. Working on Bail's aggressin theory, Okuda described the collection of subcutaneous oedema fluids from anthrax infected rabbits and the strong immunizing powers of such fluids for other rabbits. Likewise Matsumoto found the sterile oedema fluid of infected rabbits of definitely immunizing value. He concludes that the metabolic products of the B. anthracis present in such fluids are responsible for the immunity produced. and further that such aggressins are in some way comparable to bacterial toxins. Guineapigs, however, were found to be far more difficult to immunize with the aggressin. According to Salsbery recent investigations have resulted in the production of a true anthrax aggressin capable of conferring a solid immunity in different classes of domesticated animals. The aggressin is constituted by the sterilized and filtered body juices collected from the carcases of animals which have succumbed to an infection of virulent anthrax. Experiments conducted on laboratory animals as well as on sheep showed that the so-called aggressin had well marked antigenic properties, while outbreaks of anthrax amongst cattle under natural conditions could be stopped by aggressin injections.

Since the results obtained by different workers were so conflicting, it was decided to conduct some experiments with the B. anthracis both in vitro and in vivo in order to demonstrate the presence or absence of any such active metabolic products.

For this purpose a number of different liquid media was prepared. The sterile medium was then inoculated either with a fairly strong vaccine strain of anthrax, or with fully virulent cultures, and incubated at 37° C. for varying periods. After this the culture was filtered through a Seitz bacterial filter and the filtrate tested for sterility by subcultivation and injection of small animals. Should these animals succumb to anthrax, it was clear proof that filtration had been incomplete, while if smears were negative and subcultures of both the filtrate and the spleen of the dead animal sterile, death was regarded as being due to toxic action of the filtrate. The immunizing power of the filtrate was tested out on sheep and goats in the manner already described.

EXPERIMENT 8.

Six flasks each containing one litre of sterile nutrient broth of P.H. 7.6 were inoculated with 15 c.c. each of 24 hour broth culture made from a fairly strong vaccine strain, and incubated at 37° C.

Flask 1, inoculated and kept as a control.

Flask 2, 15 c.c. 24 hour broth culture of the same strain added every 14 days.

Flask 3. 200 c.c. of a 2 per cent. peptone solution added every 14 days.

Flask 4, 200 c.c. fresh broth added every 14 days.

Flask 5, 15 c.c. fresh culture + 200 c.c. broth added every 14 days.

Flask 6, 15 c.c. fresh culture + 200 c.c. 2 per cent. peptone added every 14 days.

These materials were added in an attempt to promote and continue the growth. All flasks after being incubated for five weeks, were filtered through a Seitz bacterial filter and tested for sterility.

Subsequently small animals were injected with the filtrate from each flask as follows:—

1 rabbit—5 c.c. filtrate subcutaneously.
1 guineapig—4 c.c. filtrate subcutaneously.
1 guineapig—2 c.c. filtrate subcutaneously.
1 white mouse—1 c.c. filtrate subcutaneously.
1 white mouse—.5 c.c. filtrate subcutaneously.

Of the 30 animals thus injected every one remained in good health. It was then decided after 14 days to test the immunity and the same animals now injected with virulent spore emulsion—rabbits 100 M.L.D., guineapigs 50 M.L.D., and white mice with one needle-prick from the virulent material. The result was that all the animals, excepting two mice, died from anthrax within five days after the virulent inoculation.

The material was then tested out on sheep and goats, using two sheep and two goats for each of the six filtrates, as follows:—

1 sheep—10 c.c. filtrate subcut. later tested with 500 M.L.D.
1 sheep—5 c.c. filtrate subcut. later tested with 250 M.L.D.
1 goat—10 c.c. filtrate subcut. later tested with 250 M.L.D.
1 goat—5 c.c. filtrate subcut. later tested with 100 M.L.D.

Of the 24 animals injected with filtrate not one showed any ill effect. The immunity was tested after 20 days with virulent spores ranging from 500 to 100 M.L.D.

The result was that every animal, except one sheep from flask four, died from anthrax within five days.

From this experiment it was clear that the broth filtrates were neither toxic to small animals nor did they possess any marked immunizing power for larger animals.

EXPERIMENT 9.

In this case a flask containing one litre liver broth, as prepared for the growth of B. chauveaui in the production of black-quarter filtrate, was inoculated with a young broth culture made from a reliable vaccine anthrax strain and incubated at 37° C. for 23 days, and then filtered. The sterile filtrate was injected into guineapigs and mice and also into sheep.

Two guineapigs each injected with 5 and 3 c.c. respectively, and two mice with 1 c.c. each all remained alive and healthy.

Animal.	Date Filtrate, 10/6/26.	Result.	Date Virulent Spores, 22/6/26	Result.
	c.c. subcut.		M.L.D.	
Sheep 13887.......	5	—	100	Dead, 25/6/26, Anthrax.
,, 12711.......	20	—	100	Dead, 26/6/26, Anthrax.

This filtrate therefore exhibited neither toxic action nor immunizing properties.

EXPERIMENT 10.

It was then decided to grow fully virulent anthrax organisms (*a*) in ordinary broth, (*b*) broth + 10 per cent. anthrax immune serum. This was done for a period of 30 days and the cultures filtered, and the filtrate injected into animals.

Two guineapigs were injected subcutaneously 5 and 3 c.c. respectively from each of the two filtrates. They all remained alive and were injected 14 days later with 35 M.L.D. virulent spore emulsion. They all died from anthrax within four days.

Sheep were then injected as follows:—

(a) Filtrate from plain broth culture.

Animal.	Date Filtrate, 10/6/26.	Result.	Date Virulent Spores, 22/6/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 13587.......	5	—	100	Dead, 24/6/26, Anthrax.
,, 13884.......	20	—	100	Dead, 25/6/26, Anthrax.

(b) Filtrate from broth + anthrax immune serum culture.

Animal.	Date Filtrate, 10/6/26.	Result.	Date Virulent Spores, 22/6/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 13954.......	5	—	100	Dead, 24/6/26, Anthrax.
,, 13639.......	20	—	100	Dead, 26/6/26, Anthrax.

As in Experiment 9, these filtrates were neither toxic nor did they produce immunity in sheep.

Two horses were injected subcutaneously on the left side of the neck with 10 c.c. and 5 c.c. respectively of the plain broth culture filtrate. At the same time 10 c.c. of ordinary sterile broth was injected into a third horse acting as control. The object of this experiment was to note the development of oedematous swellings frequently seen in horses after vaccination. In all three animals the temperature remained normal and not the sign of a swelling noted.

EXPERIMENT 11 (see also Expt. 3).

For this purpose the same cultures as used in Experiment 3 were filtered, and the sterile filtrates tested out on animals. This consisted of virulent anthrax grown for 30 days in pea broth, bean broth, dog broth, chicken broth, egg broth, potato broth, broth + 10 per cent. hyperimmune serum and ordinary broth. Whereas all the small animals died from anthrax following the injection of the live cultures, not a single one died from the filtrate, two guineapigs each being injected with 5 c.c. The filtrate was also tested out on two sheep, in each case receiving 15 c.c. and 5 c.c. respectively. After 14 days the immunity was tested by injection of 100 M.L.D. and 50 M.L.D. respectively. The result was that with three exceptions from different filtrates, all the sheep died from anthrax within five days. This once more proved that by growing virulent anthrax in different media neither toxic nor immunizing substances were elaborated to any appreciable extent.

EXPERIMENT 12.

In this experiment different body fluids were collected and used either as such or diluted with nutrient broth as media in which fully virulent anthrax organisms were grown for several weeks.

(*a*) A culture consisting of ordinary sterile sheep's blood in a flask inoculated with a 24 hour broth culture of virulent organisms was incubated for three weeks at 37° C. It was then diluted with two volumes of normal saline to facilitate filtration and passed through a Seitz bacterial filter. The sterile filtrate was injected into guineapigs in 5 c.c. amounts, without however producing any ill effect. It was then tested out on sheep.

Animal.	Date Filtrate, 16/9/26.	Result.	Date Virulent Spores, 29/9/26.	Result.
	c.c.		M.L.D.	
Sheep 13042.......	10	—	250	Dead, 2/10/26, Anthrax.
,, 6572.......	5	—	100	Dead, 1/10/26, Anthrax.

This filtrate therefore again showed neither toxic action nor immunizing properties.

(*b*) The fluid present in the abdominal cavity of a dog suffering from ascites was carefully drawn off and diluted with an equal volume of nutrient broth. In this medium virulent organisms were grown for three weeks and then filtered. The filtrate was injected into guineapigs without causing any symptoms or death. It was then injected subcutaneously into sheep.

Animal.	Date Filtrate, 21/10/26.	Result.	Date Virulent Spores, 1/11/26.	Result.
	c.c. filtrate.		M.L.D.	
Sheep 14260.......	5	—	100	Dead, 11/11/26, Anthrax.
,, 12376.......	10	—	100	Remained alive.

As the sheep injected with the smaller 5 c.c. dose of filtrate only died from anthrax eleven days after the injection of the virulent test dose, while the one injected with the 10 c.c. filtrate withstood the virulent test, it was decided to repeat the experiment as follows:—

Animal.	Date Filtrate, 1/12/26.	Result.	Date Virulent Spores, 13/12/26.	Result.
	c.c. filtrate.		M.L.D.	
Sheep 15171.......	10	—	100	Remained alive.
,, 15178.......	10	—	100	Dead, 20/12/26, Anthrax.

It is thus seen that in a retest one sheep again remained alive after the virulent dose, whereas the other died from anthrax seven days after the second injection. From the latter experiment it would appear that by cultivating virulent anthrax organisms in a medium consisting of ascites fluid mixed with ordinary broth some metabolic substance is elaborated which is capable of producing an immunity in a certain percentage of sheep. Except perhaps in the last mentioned experiment no definitely toxic or inert but immunizing substance could be demonstrated in any of the various liquid media in which vaccine or even virulent anthrax organisms had been cultivated for long periods. In this respect the Bacillus anthracis differs materially from such organisms as the Bacillus tetani or Bacillus chauveaui.

(*b*) Production of Immunizing of Toxic Substances in the Animal Body.

What is termed a " true anthrax aggressin " has within recent years been placed on the market. It is stated to consist of the sterilized and filtered body juices collected from the carcases of animals which have died as the result of artificial infection with anthrax. For this product is claimed the property of conferring a solid active immunity against anthrax and besides this, complete safety to vaccinated animals.

As a supply of this material had been introduced into South Africa, it was decided to ascertain experimentally its degree of safety and immunizing properties on different animals.

EXPERIMENT 13.

Subcultures made from the " aggressin " in broth and on agar all remained completely sterile. According to the directions for use, guineapigs were injected subcutaneously with the following amounts: Two guineapigs 1 c.c. each and two guineapigs 2 c.c. each. No symptoms were shown, all the animals remaining in good health. It was then decided to test the material on sheep and goats as follows:—(The dose injected corresponded with that advised by the manufacturers.)

Animal.	Date Aggressin, 10/8/26.	Result.	Date Virulent Spores.	Result.
	c.c. subcut.		24/8/26.	
Sheep 12068.......	3	—	500 M.L.D.	Dead, 30/8/26, Anthrax.
,, 10850.......	3	—	250 ,,	Dead, 27/8/26, Anthrax.
			30/8/26.	
,, 12990.......	3	—	100 M.L.D.	Dead, 2/9/26, Anthrax.
,, 12970.......	3	—	100 ,,	Dead, 4/9/26, Anthrax.
			24/8/26.	
Goat 14487.......	3	—	250 M.L.D.	Dead, 26/8/26, Anthrax.
,, 14449.......	3	—	100 ,,	Dead, 27/8/26, Anthrax.

Animal.	Date Aggressin. 10/8/28.	Result.	Date Virulent Spores.	Result.
Goat 14604.......	3	—	30/8/25. 50 M.L.D.	Dead, 2/9/26, Anthrax.
,, 14595.......	3	—	50 ,,	Dead, 2/9/26, Anthrax.

Thus of the four sheep and four goats vaccinated not a single one survived the immunity test, clearly showing that the immunity produced, if any at all, must have been very weak indeed. In none of the animals vaccinated was any temperature reaction noticed so that complete safety of the vaccine could not be disputed.

Seeing that no artificial product with reliable immunizing properties could be obtained, it was decided to ascertain whether in the animal body any such substances were elaborated after artificial infection with anthrax. With this object in view a series of experiments were conducted as follows:—

Susceptible sheep and goats were to be artificially infected with anthrax, and blood-smears examined at regular intervals. As soon as the smears revealed numerous bacilli and signs of approaching death noticed, blood from the jugular vein was to be drawn off aseptically into sterile flasks. Immediately after death, the carcase was to be carefully opened, and whatever other body fluids could be found besides the blood were to be collected in sterile vessels. Parts of the different organs were also to be collected.

The materials thus collected were to be treated in different ways:—

(*a*) Some of the body fluids, including the blood, were to be passed through Seitz bacterial filters and sterile filtrate obtained. To facilitate filtration, the blood was citrated at the moment of collection so as to prevent clotting, and afterwards diluted with two or three volumes of sterile normal saline to decrease the viscosity.

(*b*) In some flasks containing these fluids, the anthrax infection was to be killed off by chemical means. In this connection various preliminary experiments were conducted both on anthrax cultures and anthrax-infected blood. Of the various chemical substances tested out, it was found that neosalvarsan in a dilution 1 : 5,000 caused the complete sterilization of both anthrax cultures and infected blood within two hours. This was clearly proved by numerous subcultures and injections into small animals. Smears made from such material revealed marked disintegration of the organisms within 24 hours, while after a few days, only the debris of the disintegrated organisms could be made out.

Similar experiments were conducted with anilin gentian violet in various dilutions. It was found that a dilution of 1 : 2,000 killed off the anthrax infection within 24 hours, and that in the course of a few days complete disintegration had resulted. Various dilutions of Yatren were also tested but with less satisfactory results. For this series of experiments it was, therefore, decided to use unfiltered citrated blood treated either with neosalvarsan 1: 5,000 or anilin gentian violet 1 : 2,000.

(*c*) Extracts from different organs were made by macerating these up with sterile normal saline in a mortar and subsequently filtering through a bacterial filter. All these materials were then tested (1) on small animals for toxic properties, and (2) on larger animals for their immunizing value.

EXPERIMENT 14.—UNFILTERED BLOOD.

Goat 14471 was injected subcutaneously with 250 M.L.D. virulent spore emulsion. On the second day the temperature rose to 103.5° F. Twenty minutes before death blood-smears revealed numerous bacilli. Blood was collected from the jugular vein (*a*) in citrate + neosalvarsan, (b) in citrate + anilin gentian violet. Subcultures made on the following day all remained sterile. Guineapigs injected with 2 c.c. and white mice with .5 c.c. each of the two samples of blood all remained alive. The material was then tested out on sheep as follows:—

Animal.	Date Sterile Blood, 6/7/26.	Result.	Date Virulent Spores, 15/7/26.	Result.
			M.L.D.	
Sheep 12898	2 c.c. citr. + neosalvarsan blood	—	100	Remained alive.
,, 7539	5 ,, ,,	—	250	,,
,, 13821	2 c.c. citr. + an. gent. viol. blood	—	100	,,
,, 12889	5 ,, ,,	—	250	,,

As all four sheep survived the immunity test, there were definite signs that an immunity had been established. It was then decided to retest the material, using a large number of animals.

Animal.	Date Sterile Blood, 20/7/26.	Result.	Date Virulent Spores.	Result.
			M.L.D.	
Sheep 13841	2 c.c. citr. + neosalvarsan blood	—	100 8/8/26	Remained alive.
,, 13891	5 ,, ,,	—	250 16/8/26	,,
,, 13873	2 c.c. citr. + an. gent. viol. blood	—	100 16/8/26	,,
,, 9213	5 ,, ,,	—	100 8/8/26	,,
Goat 14493	2 c.c. citr. + neosalvarsan blood	—	100 16/8/26	,,
,, 14491	5 ,, ,,	—	100 8/8/26	,,
,, 14452	2 c.c. citr. + an. gent. viol. blood	—	100 8/8/26	,,
,, 14485	5 ,, ,,	—	250 16/8/26	Dead, 21/8/26, Anthrax.

Thus from the four sheep and four goats used for the retest, in which some were injected with 5 c.c. and others with 2 c.c. of the two samples of blood, only one goat succumbed from anthrax five days after the immunity test.

In some cases the immunity was tested 18 days after vaccination, while in the rest 27 days after vaccination. The goat that died was injected with 250 M.L.D. 27 days after vaccination.

Thus the preliminary evidence that an active immunity had been established by this material was proved beyond any doubt. The fact that in some cases the immunity was tested as long as 27 days after vaccination, would tend to exclude the possibility of a purely passive immunity, as e.g. shown by anthrax sera, and which generally passes off within the first week after injection.

In order to establish the effect that the neosalvarsan in the blood might have produced on the immunity test, several experiments were conducted with this drug:—

Animal.	First Injection.	Second Injection.	Result.
Sheep 11785	100 M.L.D. subcut......	1 gram neosalvarsan 48 hours later	Dead on 3rd day, Anthrax.
,, 13618	50 M.L.D. subcut......	1 gram neosalvarsan 48 hours later	Dead on 3rd day, Anthrax.
,, 13893	100 M.L.D. subcut......	1 gram neosalvarsan 24 hours later	Dead on 6th day, Anthrax.
,, 13774	50 M.L.D. subcut......	1 gram neosalvarsan 24 hours later	Dead on 4th day, Anthrax.
,, 13065	Simultaneously 100 M.L.D. + 1 gram neosalvarsan	—	Remained alive.
,, 10456	1·5 gram neosalvarsan..	100 M.L.D., 12 hours later	Dead on 3rd day, Anthrax.

Of the six sheep tested in different ways only one remained alive, viz. in the case where the virulent material and the neosalvarsan injections were made simultaneously, the first subcutaneously and the latter intravenously. As was shown in the case of sheep 10456, where 1.5 gram neosalvarsan was injected 12 hours before the virulent material, the bactericidal effect of the drug was so reduced within this short period that the animal succumbed to anthrax within three days. From these experiments it was clear that the survival of the animals treated with the blood samples and afterwards with virulent material could not have been due to any salvarsan still present in the body after 27 days.

After a period of seven weeks, this same material was again tested both for its keeping qualities as also for its thermostability. For this purpose some of the blood was pasteurized in a water bath at 60°C. for 30 minutes, while some was pasteurized at 80° C. for 30 minutes. It was then injected into sheep.

Animal.	Date Sterile Blood Injected, 20/8/26.	Result.	Date Virulent Spores, 3/9/26.	Result.
			M.L.D.	
Sheep 12038	5 c.c. citr. + neosalvarsan past. at 60° C. for 30 minutes	—	100	Remained alive.
,, 13846	5 c.c. citr. + an. gent. viol. blood past. at 60° C. for 30 minutes	—	100	,,
,, 10414	5 c.c. citr. + neosalvarsan past. at 80° C. for 30 minutes	—	100	,,
,, 10588	5 c.c. citr. + an. gent. viol. blood past. at 80° C. for 30 minutes	—	100	,,

After a period of five months this material was again tested and some pasteurized at 92° C. for 30 minutes.

Animal.	Date Sterile Blood Injected, 23/11/26.	Result.	Date Virulent Spores, 6/12/26.	Result.
			M. L. D.	
Sheep 15536	5 c.c. citr. + neosalvarsan past. at 80° C. for 30 minutes	—	100	Remained alive.
,, 15648	5 c.c. citr. + neosalvarsan blood past. at 92° C. for 30 minutes	—	100	,,
,, 15114	5 c.c. citr. + an. gent. viol. blood past. at 80° C. for 30 minutes	—	100	,,
,, 15522	5 c.c. citr. + an. gent. viol. blood past. at 92° C. for 30 minutes	—	100	Dead, 10/12/26, Anthrax

In this test one of the sheep died from anthrax four days after the virulent injection. Thus out of the 20 animals vaccinated with this material, 18 were definitely immunized even after keeping the blood for five months and then pasteurizing it up to 92° C. for 30 minutes.

EXPERIMENT 15.—FILTERED BLOOD.

In this experiment the filtered blood from sheep dead from anthrax was tested.

Sheep 12927 was injected with 250 M.L.D. and blood collected 15 minutes before its death from anthrax, blood smears showing numerous bacilli. The citrated blood was diluted with an equal volume of saline and immediately filtered. The sterile filtrate was injected into guineapigs without any ill effect. It was then injected into susceptible sheep.

Animal.	Date Sterile Filtrate, 16/9/26.	Result.	Date Virulent Spores, 29/9/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 14324.......	10	—	250	Dead, 3/10/26, Anthrax.
„ 13771.......	5	—	100	Dead, 2/10/26, Anthrax.

As these two sheep died rather unexpectedly after the immunity test, it was decided to procure a fresh sample of blood from another sheep infected with anthrax.

Sheep 13675 was injected with 250 M.L.D. virulent spores. The animal was bled a few minutes before its death on the third day. Blood-smears showed numerous anthrax bacilli. The citrated blood diluted with an equal volume of saline was again immediately filtered, and the sterile filtrate tested out on guineapigs, without any ill effect. It was then injected into sheep.

Animal.	Date Sterile Filtrate, 30/10/26.	Result.	Date Virulent Spores, 11/11/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 11671.......	2	—	250	Dead, 13/11/26, Anthrax
„ 14279.......	5	—	250	Dead, 14/11/26, Anthrax

As in the previous test, the two sheep rapidly succumbed from anthrax after the immunity test. It was thus clear that the blood from which the anthrax organisms had been separated by filtration showed no immunizing powers as compared with the immunity provoked by whole blood in which disintegration of the organisms had been brought about by chemical means. All evidence pointed to the bacillary debris in the blood as being the real immunizing substance in these experiments. The same immunizing properties, however, could not be claimed for anthrax bacilli cultivated on artificial media and disintegrated either by anilin gentian violet or by actual grinding up. (See Experiment 5.)

EXPERIMENT 16.

In this experiment the sterile filtered body juices of several animals dead from anthrax, as well as filtered organ extracts, were tested on sheep.

(*a*) A sterile saline filtrate of minced spleen obtained from goat 15225, dead from virulent anthrax, was injected into sheep.

Animal.	Date Sterile Spleen Filtrate, 30/10/26.	Result.	Date Virulent Spores, 11/11/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 13951.....	2	—	250	Dead, 3/11/26, Anthrax.
,, 13947.....	5	—	250	Dead, 14/11/26, Anthrax.

(*b*) Sterile filtered bile obtained from the same goat was also tested on sheep.

Animal.	Date Sterile Filtered Bile, 30/10/26.	Result.	Date Virulent Spores, 11/11/26.	Result.
	c.c. bile filtrate.		M.L.D.	
Sheep 14326.....	2	—	100	Remained alive.
,, 13725.....	5	—	100	Dead, 12/11/26, Anthrax.

(*c*) Sterile filtered peritoneal fluid obtained from goat 14391, dead from virulent anthrax, was injected into sheep.

Animal.	Date Sterile Fluid, 16/9/25.	Result.	Date Virulent Spores, 29/9/26.	Result.
	c.c. subcut.		M.L.D.	
Sheep 12201.......	10	—	100	Dead, 3/10/26, Anthrax.
,, 13992.......	5	—	100	Remained alive.

On repeating the latter two experiments both animals died after the virulent test. Thus, as in the case of the filtered blood, the other body fluids and organ extracts showed no definite immunizing powers.

EXPERIMENT 17.—OEDEMA FLUID.

A number of horses vaccinated with a certain fairly weak batch of anthrax spore vaccine developed well-marked subcutaneous, oedematous swellings. It was decided to collect some of this oedema fluid and test it out on animals. Horse 18028, showing a severe oedema of the lower cervical and sternal reigons following vaccination, was used for supplying the oedema fluid.

The clear fluid was drawn off into a sterile flask. Subcultures made from the fluid all remained sterile. Guineapigs injected with 5 c.c. each of the pure oedema fluid all remained alive.

Animal.	Date Oedema Fluid, 30/8/26.	Result.	Date Virulent Spores, 13/9/26.	Result.
	c.c. oed. fluid.		M.L.D.	
Sheep 13909.....	10	—	250	Dead, 16/9/26, Anthrax.
,, 13748.....	10	—	100	Remained alive.
,, 13069.....	5	—	250	Dead, 18/9/26, Anthrax.
,, 12695.....	5	—	100	Dead, 17/9/26, Anthrax.

Thus out of the four sheep injected with as much as 10 c.c. of the pure fluid, three died from anthrax after the immunity test, showing that the immunizing power, if any, was decidedly very weak. At the same time this fluid was also injected into two non-vaccinated horses, the object being to note the possible development of similar swellings. Horse 17827 was injected with 5 c.c. and horse 17700 with 10 c.c. both subcutaneously on the side of the neck. The animals were examined daily for 12 days and temperature records kept. During this time neither of the animals showed any temperature disturbances nor was there any swelling noticed.

EXPERIMENT 18.

The object of this experiment was to ascertain the effect of various non-living anthrax products on the course of an anthrax infection in guineapigs. Guineapigs were injected subcutaneously with equal amounts of a definite dilution of vaccine anthrax pores. In some of these, left as contols, the time of death was carefully noted, while the others were injected simultaneously with the various anthrax products, which by themselves showed no harmful effects on these animals. Again the time of death was noted.

All animals injected on the 17/9/26.

(*a*) *Controls.*

1 guineapig—1 c.c. of .01 dilution spores—dead in four days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores—dead in five days, Anthrax.

(*b*) Vaccine spores + sterile broth filtrate obtained from 30 days old virulent anthrax broth culture.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in four days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in four days, Anthrax.

(*c*) Vaccine spores + oedema fluid from horse 18028 showing anthrax swellings after vaccination.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. oedema fluid—dead in four days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. oedema fluid—dead in five days, Anthrax.

(*d*) Vaccine spores + sterile filtrate obtained from 30 day old blood culture of virulent Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in four days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in four days, Anthrax.

(*e*) Vaccine spores + sterile filtrate obtained from anthrax blood of sheep 12927.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in four days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in six days, Anthrax.

(*f*) Vaccine spores + sterile filtered peritoneal fluid from goat 14391—dead from Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in three days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. filtrate—dead in four days, Anthrax.

(*g*) Vaccine spores + imported sterile Anthrax aggressin.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. aggressin—dead in three days, Anthrax.

1 guineapig—1 c.c. of .01 dilution spores + 2 c.c. aggressin—dead in four days, Anthrax.

From these experiments it is clear that the different anthrax products used had no effect on the course of an anthrax infection in guineapigs when injected simultaneously with the live spores. In none of the cases was death definitely hastened or delayed, since all the animals died practically on the same day as the controls did. It should be noted that the spores used in this experiment were repeatedly washed and centrifugalized in order to free them from any adherent exo-products.

From all the experiments recorded in this section the following points were clearly evident:

(*a*) In every instance the metabolic products elaborated by the B. anthracis, when cultivated in various liquid media for long periods and then filtered off, failed to reveal any toxic action in small animals or immunizing effect in sheep and goats.

(*b*) Various body juices, including blood, collected from anthrax-infected animals and passed through bacterial filters were neither toxic nor of immunizing value.

(*c*) Anthrax blood in which disintegration of the B. anthracis had been brought about by chemical means possessed definite immunizing properties which resisted heating at 92° C. for 30 minutes. The same qualities could not be claimed for disintegrated organisms after cultivation on artificial media.

(*d*) No soluble filterable "aggressin" could be demonstrated in any of the experiments. The immunizing substance present in anthrax blood was not found to be filterable, i.e. it is probably very closely associated with the organism in the blood, although it was not found in the case of organisms cultivated on artificial media.

4. COMPARATIVE SAFETY AND IMMUNITY EXPERIMENTS WITH ANTHRAX SPORE VACCINE.

Based on the results of various experiments conducted, Besredka has within recent years announced an original theory as to the mechanism of infection and immunity in anthrax. According to this theory, animals which are highly susceptible to anthrax can only become infected through the skin, which is the only organ capable of acting as the portal of entry for the anthrax organism. Where an infection of the skin was definitely excluded, a fatal artificial infection by any other route could not be brought about even in the case of highly susceptible animals such as guineapigs.

Further, successful immunization of susceptible animals is dependent on the primary immunity of the skin. Thus immunization of the skin is essential and sufficient in order to render animals immune to anthrax.

It is for these reasons that Besredka strongly advocates skin vaccination as the rational mode of immunizing animals against anthrax. Laboratory animals such as rabbits and guineapigs which by the usual methods of vaccination were very difficult to immunize could by cuti-vaccination be protected. These findings of Besredka have resulted in numerous investigators focusing attention on cuti-vaccination and cuti-immunity. The results obtained by different workers are, however, again markedly conflicting. While the findings of some are wholly in accordance with those of Besredka, others were able to substantiate only certain points in the original theory. Some investigators, on the other hand, have failed to confirm any of Besredka's findings. Of the various authors who have confirmed the results obtained by Besredka, the following may be mentioned:—Velu believes that extensive vaccination of animals in Morocco has shown that a single intradermal injection of vaccine is sufficient to provoke a strong and lasting immunity, even where no thermal or local reaction follows vaccination. The method of vaccination is practical and the immunity lasting six to seven months.

According to Nevodov, horses and cattle can be successfully immunized by cuti-vaccination, using the vaccine 1 only. No complications are noted, while animals may be worked immediately after vaccination.

From numerous experiments Brocq-Rousseau concludes that cuti-vaccination of horses leads to a strong and rapidly forming immunity without, however, any untoward complications.

Similarly Balteano, Plotz, Nicolas, Stoicescu, Vallee, Hruska, Kudrjavzev, Baliberdin, Rossi, Urbain and Rossi, were able to confirm the findings of Besredka.

Several authors, although partly in agreement with Besredka's theory, find that the skin is not the only organ susceptible to anthrax.

Thus Combiesco, Popesco, Gratia, Kritschewsky and Brussin, and more recently Bautz and Amiraslanow, maintain that animals may be fatally infected by routes other than the skin. Furthermore, numerous experiments have shown that infection through the skin is less certain than by other routes, i.e. the skin is actually less susceptible than other tissues.

From various experiments conducted, Viljoen, Curson and Fourie found the skin no more susceptible than any other organ, and, further, the immunity following subcutaneous vaccination more reliable than that following cuti-vaccination. According to Gusman, Neri, Pazewicz, Lanfranchi and Casalotti, their experiments did not confirm Besredka's findings, i.e. cuti-vaccination of laboratory animals was not followed by reliable immunity against anthrax.

Seeing that there exists a wide difference of opinion as to the relative susceptibility of animals to different routes of infection as well as the merits of different methods of vaccination, a lengthy series of experiments were conducted with the following objects in view:—

(*a*) To attempt the artificial infection of small animals with anthrax in different ways, at the same time excluding as far as possible infection in other ways.
(*b*) To attempt the immunization of small animals by different methods of vaccination.
(*c*) To compare safety and immunity conferred by anthrax spore vaccine on sheep and goats by different methods of vaccination.
(*d*) To note the clinical reaction produced in horses following various methods of vaccination.

In all these experiments the vaccine consisted of a glycerinized spore emulsion of a batch of anthrax vaccine, the safety and immunizing properties of which had been carefully ascertained beforehand. The vaccine in each case was prepared by cultivating the attenuated organisms on nutrient agar and collecting the growth as soon as sporulation was practically complete. Subsequently the spore emulsion preserved in an equal weight of glycerine was tested out on guineapigs and rabbits by subcutaneous injection of various fractions of one cubic centimetre, and the lethal effect noticed. Should the rabbits survive, the material was tested out on sheep and goats in amounts ranging from 20 c.c. to .001 c.c. of the emulsion by subcutaneous injection.

After a period of two to three weeks the immunity was ascertained by the subcutaneous injection of virulent anthrax spore emulsion. This constitutes the routine procedure for the testing of different batches of anthrax vaccine before issue.

EXPERIMENT 19.

In this experiment only guineapigs were used. Precautions were taken to select animals that had not been in anthrax experiments previously. Where it was desired to exclude, as far as possible, skin contamination with anthrax material in the course of an injection, long hypodermic needles of very small bore were used. The point of the needle was introduced under the skin and carried as far as possible from the skin puncture before the injection was made. On withdrawing the needle, the edge of the skin at the puncture was immediately disinfected as thoroughly as possible by introducing the points of a very fine pincet rolled in cotton wool and steeped in a 10 per cent. solution of tincture of iodine. By twisting the points of the pincet in the skin puncture, the edges were probably immediately and thoroughly disinfected. This procedure was adopted in every case where skin contamination was not desired. Guineapigs were injected with a definite dilution of a fairly weak vaccine known to kill them in that dilution after the usual subcutaneous inoculation. Animals that survived the first injection were, afterwards injected subcutaneously with a stronger vaccine and which was regarded as the immunity test.

(*a*) *Intraperitoneally—preventing skin contamination.*

1 guineapig—.01 c.c.—dead after seven days Anthrax.
1 guineapig—.01 c.c—dead after eight days Anthrax.

(*b*) *Deep intramuscular—preventing skin contamination.*

1 guineapig—.01 c.c.—dead after six days Anthrax.
1 guineapig—.01 c.c.—dead after six days Anthrax.

(*c*) *Subcutaneous—preventing skin contamination.*

1 guineapig—.01 c.c.—dead after seven days Anthrax.
1 guineapig—.01 c.c.—survived first injection.

Injected 14 days later with .2 c.c. stronger vaccine. Dead four days later Anthrax.

(*d*) *Scarification of skin and rubbing on of Vaccine.*

1 guineapig—.01 c.c.; 1 guineapig—.01 c.c.: Both survived first injection. Injected 14 days later with .2 c.c. stronger vaccine. One dead three days later Anthrax.

(*e*) *Intratracheally—preventing skin contamination.*

1 guineapig—.01 c.c.; 1 guineapig—.01 c.c.: Both survived first injection. Injected 14 days later with .2 c.c. stronger vaccine. Both dead three days later, Anthrax.

(*f*) *Per os. On to the back of the tongue.*

1 guineapig—.1 c.c.; 1 guineapig—.1 c.c.: Both survived first dose. Injected subcutaneously 14 days later with .2 c.c. stronger vaccine. Both dead four days later Anthrax.

Controls.—Two guineapigs injected subcutaneously in the ordinary way with .01 c.c. each of the first weaker vaccine died in four and five days respectively from anthrax.

Two guineapigs injected subcutaneously with .2 c.c. each of the second stronger vaccine both died in four days from anthrax.

Thus in the above experiments guineapigs were readily killed by intraperitoneal, intramuscular, and subcutaneous injections of a weak vaccine, while all the guineapigs survived the intratracheal, oral, and intradermal by scarification, administration of the weak vaccine. These animals, however, all died after the test injection of a stronger vaccine 14 days later.

The above experiment was repeated, using smaller doses of the weak first vaccine, in order to lose fewer animals and to be able to test the immunity developed by means of a second injection of the same stronger vaccine.

(*a*) *Intraperitoneal—preventing skin contamination.*

1 guineapig—.001 c.c.; 1 guineapig—.001 c.c.: Both survived first injection. Injected 14 days later with .2 c.c. stronger vaccine. Both died four days later from anthrax.

(*b*) *Intramuscular—preventing skin contamination.*

1 guineapig—.001 c.c.—dead six days later from Anthrax.

1 guineapig—.001 c.c.—survived first injection. Injected 14 days later with .2 c.c. stronger vaccine. Remained alive.

(*c*) *Subcutaneous—preventing skin contamination.*

1 guineapig—.001 c.c.—dead after nine days from Anthrax.

1 guineapig—.001 c.c.—Survived first injection. Injected 14 days later with .2 c.c. stronger vaccine. Dead four days later from Anthrax.

(*d*) *Scarification of skin and rubbing on of vaccine.*

1 guineapig—.001 c.c.— dead seven days afterwards Anthrax.

1 guineapig—.001 c.c.—survived injection. Injected 14 days later with .2 c.c. stronger vaccination. Dead three days later from Anthrax.

(*e*) *Intratracheal—preventing skin contamination.*

1 guineapig—.001 c.c.; 1 guineapig—.001 c.c.: Both survived first injection. Injected 14 days later with .2 c.c. stronger vaccine. Both dead four days later Anthrax.

(*f*) *Per os—on to the back of the tongue.*

1 guineapig—.01 c.c.; 1 guineapig—.01 c.c.: Both survived first dose. Injected subcutaneously 14 days later with .2 c.c. stronger vaccine. Both dead four days later Anthrax.

A third immunity test was conducted in which case a larger number of guineapigs were vaccinated, with the same first vaccine in a dilution of 1:300 as issued to stockowners. The immunity was subsequently tested with .01 c.c. instead of .2 c.c. of the second stronger vaccine, by subcutaneous injection.

(*a*) *Subcutaneous vaccination, preventing skin contamination.*

Animal.	Date Vaccine, 3/5/27.	Result.	Date Stronger Vaccine, 16/5/27.	Result.
	c.c. (dil. 1 : 300).		c.c. subcut.	
1 guineapig........	1	Dead, 11/5/27, not Anthrax	—	—
1 ,, ...	1	Alive..........	0·01	Dead, 19/5/27, Anthrax.
1 ,, ...	1	,, ...	0·01	Dead, 20/5/27, Anthrax.
1 ,, ...	1	,, ...	0·01	Dead, 20/5/27, Anthrax.
1 ,, ...	1	,, ...	0·01	Remained alive.

(*b*) *Deep intramuscular vaccination, preventing skin contamination.*

Animal.	Date Vaccine, 3/5/27.	Result.	Date Stronger Vaccine, 16/5/27.	Result.
	c.c. (dil. 1 : 300).		c.c. subcut.	
1 guineapig........	1	Dead, 9/5/27, Anthrax	—	—
1 ,, ...	1	Dead, 11/5/27, Anthrax	—	—
1 ,, ...	1	Alive..........	0·01	Remained alive.
1 ,, ...	1	,, ...	0·01	,,
1 ,, ...	1	,, ...	0·01	,,

(*c*) *Intradermal injection of Vaccine.*

Animal.	Date Vaccine, 3/5/27.	Result.	Date Stronger Vaccine, 16/5/27.	Result.
	c.c. (dil. 1 : 30).		c.c. subcut.	
1 guineapig........	0·1	Alive..........	0·01	Dead, 20/5/27, Anthrax.
1 ,, ...	0·1	,, ...	0·01	Dead, 20/5/27, Anthrax.
1 ,, ...	0·1	,, ...	0·01	Dead, 23/5/27, Anthrax.
1 ,, ...	0·1	,, ...	0·01	Remained alive.
1 ,, ...	0·1	,, ...	0·01	,,

From this retest it is seen that out of five guineapigs vaccinated subcutaneously, one died after vaccination but not from anthrax, while following the immunity test three out of four died from anthrax. Of those injected intramuscularly two out of the five died from anthrax after vaccination, while all three the others withstood the immunity test. Of the five vaccinated intradermally three died after the immunity test.

It should be noted that in the original experiment as well as in the retests the same vaccine was used, only in different dilutions, while the same stronger vaccine was used for all the immunity tests. The following points were clear:—

(*a*) Guineapigs could be killed by intraperitoneal injections of vaccine anthrax, while in surviving cases no immunity could be demonstrated.

(*b*) Guineapigs died from anthrax after deep intramuscular injection of vaccine, while in surviving cases the immunity was fairly definite.

(*c*) Guineapigs were killed by subcutaneous injection of vaccine, while in surviving cases no immunity could be demonstrated.

(*d*) By scarification of the skin or intradermal vaccination, some guineapigs died from anthrax, while in surviving cases the immunity was uncertain.

(*e*) By intratracheal injections of vaccine, no deaths occurred, although no immunity could be demonstrated afterwards, since all the animals died after the test injection.

(*f*) By the oral administration of ten times the amount of vaccine used in the other tests, none of the guineapigs died, although no immunity could be shown afterwards.

Thus, except for the intratracheal and oral administration of anthrax vaccine, guineapigs could be easily killed by the different routes, while from the immunity tests the intramuscular vaccination would seem the most promising, while the results of intradermal vaccination were doubtful.

Comparative Susceptibility and Immunity Experiments conducted on Sheep and Goats.

This series of experiments was carried out with the object of:—

(*a*) Ascertaining the differences in susceptibility to the vaccine and the degree of immunity developed in sheep, and especially in goats, following different methods of vaccination with spore vaccine.

(*b*) Ascertaining the merits of cuti-vaccination.

EXPERIMENT 20.

The vaccine used was the same as that employed on the guineapigs in the previous experiment. It was prepared from a fairly weak vaccine strain and issued for use in goats in a dilution of 1:300. In the routine tests on guineapigs, it killed them in a dilution of .01 c.c., while .001 c.c. proved safe. Tested out on sheep and goats all animals remained alive, although in the immunity test two out of the six sheep died as well as three out of 14 goats, showing that the immunizing power of the vaccine was not very strong.

The main aim of these tests was to compare the safety and immunity following (*a*) subcutaneous and (*b*) cuti-vaccination, but since the intramuscular vaccination had given the most encouraging results in guineapigs, it was decided to include this method of vaccination in the tests. As in the guineapig experiments, strict precautions were taken in order to minimise skin contamination with the vaccine when injected subcutaneously or intramuscularly. The injection was thus made as far as possible from the puncture of the skin, while the wound was immediately disinfected with the pincet and cotton wool swab soaked in 10 per cent. tincture of iodine solution. The vaccination in all cases was carried out on the inside of the thigh, while for the subsequent immunity test the inside of the other thigh was used. Temperature records were kept of all the animals.

The object in using the weak vaccine was to note the differences, if any, in the degree of immunity since with a stronger vaccine it is conceivable that these differences might quite easily have been masked. For the immunity tests a standard fully virulent spore emulsion of known strength was used. In this case the injections, unless otherwise stated, were all made subcutaneously into the other thigh of the animal.

A. All vaccine injections made *subcutaneously*, preventing skin contamination :—

Animal.	Date Vaccine, 5/4/27.	Result.	Date Virulent Spores.	Dose Virulent Spores.	Result.
	c.c. (dil. 1 : 300).			M.L.D.	
Sheep 15790......	1	—	19/4/27	500	Dead, 25/4/27, Anthrax.
,, 15546......	1	—	25/4/27	500	Remained alive.
,, 15584......	1	—	,,	500	Dead, 29/4/27, Anthrax.
,, 15776......	1	—	,,	250	Dead, 29/4/27, Anthrax.
,, 5474......	1	—	,,	750	Dead, 29/4/27, Anthrax.

B. All vaccine injections made *intramuscularly*, preventing skin contamination :—

Animal.	Date Vaccine, 5/4/27.	Result.	Date Virulent Spores.	Dose Virulent Spores.	Result.
	c.c. (dil. 1 : 300).			M.L.D.	
Sheep 10897......	1	—	19/4/27	500	Remained alive.
,, 15575......	1	—	25/4/27	500	,,
,, 15757......	1	—	,,	500	,,
,, 15467......	1	—	,,	250	Dead, 28/4/27, Anthrax.
,, 15643......	1	—	,,	750	Remained alive.

C. Vaccine applied to skin after scarification of a patch 1½ inch square :—

Animal.	Date Vaccine, 5/4/27.	Result.	Date Virulent Spores.	Dose Virulent Spores.	Result.
	c.c. (dil. 1 : 75).			M.L.D.	
Sheep 15649......	0·25	—	19/4/27	500	Dead, 23/4/27 Anthrax.
,, 15547......	0·25	—	25/4/27	500	Dead, 27/4/27, Anthrax.
,, 10920......	0·25	—	,,	500	Dead, 29/4/27, Anthrax.
,, 15460......	0·25	—	,,	250	Dead, 29/4/27, Anthrax.
,, 15458......	0·25	—	,,	750	Remained alive.

Thus, out of the five sheep vaccinated subcutaneously four succumbed to anthrax after the immunity test, and likewise four of the five in which the vaccine was applied on the scarified skin, died after the immunity test. Of the five vaccinated intramuscularly, only one died. As mentioned before the vaccine was definitely weak, but still the differences in the results following different methods of vaccination were clearly shown, as was the case with the guineapigs, where intramuscular vaccination also appeared the most promising. No difference was noted in the results of cuti-vaccination as compared to subcutaneous injection.

It should be mentioned that none of the sheep injected subcutaneously or by scarification showed any temperature reaction after the vaccination, whereas of those injected intramuscularly two showed a welll marked rise in temperature up to 106.4° F. on the third day after vaccination, while two showed a rise to 103.8° F. and the fifth only a very slight rise. Thus, the reaction produced by intramuscular injection, as gauged by the temperature charts, was clearly more marked than with the other two methods.

A retest of the above experiments was carried out on nine more sheep as follows:—

A. All vaccine injections made *subcutaneously*, preventing skin contamination:—

Animal.	Date Vaccine, 28/5/27.	Result.	Date Virulent Spores, 11/6/27.	Result.
	c.c. (dil. 1 : 300).		M.L.D.	
Sheep 16157.......	1	—	250	Dead, 19/6/27, Anthrax.
,, 15778.......	1	—	500	Remained alive.
,, 16170.......	1	—	750	,,

B. All vaccine injected *intramuscularly*, preventing skin contamination:—

Animal.	Date Vaccine, 28/5/27.	Result.	Date Virulent Spores, 11/6/27.	Result.
	c.c. (dil. 1 : 300).		M.L.D.	
Sheep 16159.......	1	—	250	Remained alive.
,, 10912.......	1	—	500	,,
,, 16164.......	1	—	750	,,

C. All vaccine injected *intradermally*.

Animal.	Date Vaccine, 28/5/27.	Result.	Date Virulent Spores, 11/6/27.	Result.
	c.c. (dil. 1 : 75).		M.L.D.	
Sheep 15769.......	0·25	—	250	Dead, 13/6/27, Anthrax.
,, 15831.......	0·25	—	500	Remained alive.
,, 10918.......	0·25	—	750	,,

From the retest it is seen that one out of the three vaccinated subcutaneously as well as one out of the three vaccinated into the skin succumbed to the immunity test, while those injected intramuscularly remained alive.

A third test on sheep was carried out with the same vaccine although the immunity test dose was increased up to 3,000 M.L.D. The object of the experiment was to note the quantity of virulent spore emulsion necessary to break the immunity in each case:—

A. All vaccine *subcutaneously*, preventing skin contamination:

Animal.	Date Vaccine, 11/6/27.	Result.	Date Virulent Spores, 25/6/27.	Result.
	c.c. (dil. 1 : 300).		M.L.D.	
Sheep 15538.......	1	—	1,500	Dead, 28/6/27, Anthrax.
,, 16142.......	1	—	2,000	Dead, 28/6/27, Anthrax.
,, 16151.......	1	—	2,500	Remained alive.
,, 16139.......	1	—	3,000	,,

B. All vaccine *intramuscularly*, preventing skin contamination:

Animal.	Date Vaccine, 11/6/27.	Result.	Date Virulent Spores, 25/6/27.	Result.
	c.c. (dil. 1 : 300).		M.L.D.	
Sheep 15391.......	1	—	1,500	Remained alive.
,, 15781.......	1	—	2,000	,,
,, 15807.......	1	—	2,500	,,
,, 15651.......	1	—	3,000	,,

C. All vaccine injected *intradermally*.

Animal.	Date Vaccine, 11/6/27.	Result.	Date Virulent Spores, 25/6/27.	Result.
	c.c. (dil. 1 : 75).		M.L.D.	
Sheep 15796.......	0·25	—	1,500	Remained alive.
,, 15792.......	0·25	—	2,000	Dead, 28/6/27, Anthrax.
,, 15810.......	0·25	—	2,500	Remained alive.
,, 15779.......	0·25	—	3,000	Dead, 28/6/27, Anthrax.

Thus, two out of four vaccinated subcutaneously, and another two vaccinated intradermally, succumbed after the immunity test, while all four injected intramuscularly remained alive.

Seeing that the intradermal vaccination produced no better immunity than the subcutaneous method, the former was discontinued. Comparative tests were then conducted on goats, some vaccinated subcutaneously and others intramuscularly.

Comparative Immunity Tests on Goats.

EXPERIMENT 21.

The same vaccine which was employed in the previous experiments was also used on goats as follows:—

A. Vaccine injected *subcutaneously*, preventing skin contamination:

Animal.	Date Vaccine, 12/8/27.	Result.	Date Virulent Spores, 29/8/27.	Result.
	c.c. (dil. 1 : 300).		M.L.D.	
Goat 17951........	1	—	500	Dead, 31/8/27, Anthrax.
,, 17967........	1	—	500	Dead, 31/8/27, Anthrax.
,, 17952........	1	—	1,000	Remained alive.
,, 17970........	1	—	500	Dead, 31/8/27, Anthrax.
,, 17947........	1	—	1,000	Remained alive.

B. Vaccine injected *intramuscularly* preventing skin contamination:

Animal.	Date Vaccine, 12/8/27.	Result.	Date Virulent Spores, 29/8/27.	Result.
	c.c. (dil. 1 : 300).		M.L.D.	
Goat 17975........	1	—	500	Remained alive.
,, 17972........	1	—	500	,,
,, 17963........	1	—	1,000	,,
,, 17968........	1	—	500	,,
,, 17962........	1	—	1,000	,,

Result.—Out of the five goats vaccinated subcutaneously, three died from anthrax after the immunity test, while all five the goats vaccinated intramuscularly survived the immunity test.

It was then decided to ascertain the degree of safety of a strong cattle vaccine for goats when injected subcutaneously in the one case, and deep intramuscularly in the other. For this purpose 1 c.c. of a 1 in 10 dilution of a strong cattle vaccine was used. The vaccine regularly killed guineapigs in a dilution of 1 in 1,000.

A. Vaccine injected *subcutaneously*, preventing skin contamination.

Animal.	Date Vaccine, 23/8/27.	Result.	Date Virulent Spores, 15/9/27.	Result.
	cc. (dil. 1 : 10).		M.L.D.	
Goat 17984........	1	Dead, 29/8/27, Anthrax	—	—
,, 17974........	1	Alive..........	1,500	Remained alive.
,, 17989........	1	,, ...	1,500	,,
,, 17961........	1	Dead, 1/9/27, Anthrax	—	—
,, 17944........	1	Alive..........	1,500	Remained alive.
,, 17976........	1	,, ...	1,500	,,
,, 17945........	1	Dead, 26/8/27, Anthrax	—	—
,, 17971........	1	Dead, 31/8/27, Anthrax	—	—
,, 17941........	1	Dead, 27/8/27, Anthrax	—	—
,, 17960........	1	Alive..........	1,500	Remained alive.

B. Vaccine injected *intramuscularly*, preventing skin contamination.

Animal.	Date Vaccine, 23/8/27.	Result.	Date Virulent Spores, 15/9/27.	Result.
	c.c. (dil. 1 : 10).		M.L.D.	
Goat 17964........	1	Alive..........	1,500	Remained alive.
,, 17966........	1	,, ...	1,500	,,
,, 17982........	1	,, ...	1,500	,,
,, 17981........	1	Dead, 29/8/27, Anthrax	—	—
,, 17965........	1	Alive..........	1,500	Remained alive.
,, 17942........	1	,, ...	1,500	,,
,, 17955........	1	,, ...	1,500	,,
,, 17977........	1	,, ...	1,500	,,
,, 17969........	1	Dead, 27/8/27, Anthrax	—	—
,, 17987........	1	Dead, 26/8/27, Anthrax	—	—

Result.—Of the ten goats vaccinated subcutaneously, five died from anthrax a few days after vaccination. Of the ten goats injected intramuscularly, three died from anthrax after vaccination. Of the 12 surviving goats, every single one withstood the immunity test of 1,500 M.L.D. virulent spores, showing that a solid immunity had been established by this strong vaccine irrespective of the method of vaccination. With reference to the relative safety of vaccination as shown by the two results, it would appear that fewer deaths might be expected from intramuscular vaccination than from subcutaneous injections.

The temperature reaction following vaccination was equally well marked in both lots of animals.

COMPARATIVE SUSCEPTIBILITY AND IMMUNITY EXPERIMENTS ON HORSES.

Since of the various species of domesticated animals usually vaccinated against anthrax, horses seem to be the most susceptible to the development of subcutaneous oedematous swellings following vaccination, a series of experiments were conducted with the object of (*a*) ascertaining what factors are involved in the production of swellings; (*b*) the relative value of different methods of vaccination as far as the incidence of swellings was concerned. As stated before, although the horse shows well-marked susceptibility to anthrax under natural conditions, it is very difficult to cause a fatal anthrax infection by the administration of cultures. It is for this reason that immunity tests were not carried out on a large scale, since these could not be regarded as being very reliable.

EXPERIMENT 22.

In order to ascertain the cause of the swellings following vaccination, a number of different materials was injected subcutaneously into horses. It was reasonable to suspect either some non-living toxic material elaborated by the anthrax organism in the artificial media and collected with the spores in the preparation of the vaccine, or, alternately, its elaboration in the animal body at the seat of inoculation. As a second possibility, had to be consideerd the harmful effect that the organism itself might have on the live tissues, especially the blood vessels of the part, and allowing an increased capillary permeability.

(*a*) *Injection of broth filtrates.*

As mentioned in the latter part of Experiment 10 (which see), two horses were injected subcutaneously on the side of the neck with 10 c.c. and 5 c.c. respectively of a sterile broth filtrate prepared by growing fully virulent anthrax organisms in broth for 30 days. As control a third horse was injected with 10 c.c. ordinary sterile nutrient broth. In all three animals the temperature remained undisturbed and not a sign of a swelling was noted. Toxic substances elaborated in the broth were, therefore, excluded.

(*b*) *Fluid from Agar.*

It was noticed that a small amount (1-2 c.c.) of condensation fluid collected on the surface of the nutrient agar after this had set in the flat-bottomed Fernbach flasks prior to inoculation with the broth cultures. As some of this fluid at any rate would be collected with the spores, and thus be present in the vaccine, it was poured off from a number of agar containing flasks and injected subcutaneously into two horses, 5 c.c. each, on the side of the neck. They were kept under observation for seven days without, however, showing any swelling.

(*c*) *Washed Vaccine Spores.*

A certain, fairly weak batch of vaccine was injected in a dilution 1 in 100 in the ordinary way subcutaneously into 16 horses. Of these, seven showed well-marked oedematous swellings and six slight swellings within a few days after vaccination. The remaining three did not show any swelling. Seeing that this vaccine definitely produced swellings in horses, a small amount of the concentrated glycerinized spore emulsion was repeatedly washed in saline and centrifuged. The washed spores were then as near as possible again diluted to 1 : 100 and injected into horses 1 c.c. each subcutaneously on the side of the neck as follows:—

Animal.	Vaccination.	Result.
	c.c. washed spores subcut.	
Horse 17605	1	Small diffuse swelling on 4th day, disappeared on the 7th day.
,, 17970	1	Small swelling on 5th day, disappeared on the 8th day.
,, 17314	1	Negative.
,, 17628	1	,,
,, 17717	1	,,
,, 69	1	,,
,, 17793	1	,,
,, 17613	1	,,
,, 17689	1	,,
,, 17541	1	,,

Thus, out of the ten horses vaccinated with washed spores subcutaneously, two only showed small swellings at the site of injection.

At the same time ten more horses were shaved on the side of the neck and a small patch scarified. Ordinary glycerinized emulsion from the same batch as used above (and which had produced swellings in 13 out of 16 horses) was rubbed in to the scarified parts .1 c.c. of a 1 : 10 dilution. In none of these ten animals was there any sign of the development of a swelling.

In order to check the results obtained in the first test where the animals showed swellings, five control horses were again vaccinated subcutaneously with the same ordinary glycerinized emulsion again in a dilution of 1 : 100. The result of the retest was that no swelling developed in any of the five horses. It should be mentioned that the same aseptic precautions in all these experiments were observed throughout, i.e. the skin was carefully disinfected in each case, and all the instruments boiled. The only difference in these two experiments was that the first lot of 16 horses that showed swellings were inoculated under veld conditions, the animals running in a paddock day and night. The experiment was conducted in September, 1926. The weather was fairly warm throughout, and no rain fell on the animals. The other animals vaccinated with the washed spores, as well as those done by scarification and the last five controls injected subcutaneously, were stabled at night and allowed out into a paddock during the day.

EXPERIMENT 23.

It was then decided to ascertain the effect on horses of a strong batch of cattle vaccine that had killed a goat and also guineapigs in dilutions up to 1 in 1,000.

(*a*) Ordinary glycerinized spore emulsion dilution 1:1,000 injected subcutaneously on side of neck 1 c.c. each.

Animal.	Result.
Horse 17714	Small flat diffuse swelling on 4th day, disappeared 6th day.
,, 17876	Negative.
,, 17636	,,

(*b*) Ordinary glycerinized emulsion dilution 1:100 rubbed on to scarified patch .1 c.c. each.

Animal.	Result.
Horse 17779	Negative.
,, 17441	,,
,, 17705	,,

(*c*) Twenty-four hour broth culture prepared from the above spore emulsion, repeatedly washed in saline and centrifuged. Suspension of washed bacilli only, in dilution 1:100, was injected subcutaneously 1 c.c. each.

Animal.	Result.
Horse 17444	Negative.
,, 17965	,,
,, 17701	,,

(*d*) Ordinary spore emulsion repeatedly washed in saline and centrifuged. Suspension of washed spores only in a dilution of 1: 100 injected subcutaneously 1 c.c. each.

Animal.	Result.
Horse 17536	Large round swelling, 6 × 4″, running down and painful on the 3rd day, subsided on 6th day.
,, 17620	Long running down swelling, 6 × 2″, on 3rd day subsided on 7th day.
,, 17540	Negative.

Thus, out of the 12 horses inoculated in different ways, some with ordinary emulsion and others with washed spores and washed bacilli, only the two of the three animals injected subcutaneously with the washed spores showed large and well-marked swellings. The others, except for the one horse 17714 injected with the ordinary glycerinized emulsion subcutaneously and which showed a small round swelling on the 4th day, all remained in normal health.

Although it is very difficult to interpret these results, one point seems certain, that the washed spores freed from all extracellular material are capable of producing swellings.

In Experiment 17 (which see), it was mentioned that the clear fluid collected from the oedematous swelling of Horse 18028 following vaccination was again injected into two other horses in 5 and 10 c.c. amounts without, however, producing the slightest swelling in them. From the results obtained with the washed spores it would seem that swellings could be produced without the presence of toxic substances in the vaccine. Furthermore, such substances could not be detected in the oedema fluid as tested out on other horses.

EXPERIMENT 24.

The object of this experiment was to ascertain and compare swellings in horses vaccinated (*a*) subcutaneously, (*b*) intramuscularly with a fairly strong anthrax spore vaccine. All the horses were injected in the side of the neck.

(*a*) *Subcutaneous injection.*—Of the 15 horses injected 1 c.c. each of a dilution 1:500, eight showed well-marked swellings, four slight swellings and three were negative.

(*b*) *Intramuscular injection*, preventing skin contamination by swabbing needle wound with tincture of iodine.—Of the 15 horses injected 1 c.c. each of a dilution 1:500, eight showed well-marked swellings, three slight swellings and five were negative.

It is thus seen that the number showing swellings were more or less equally divided between the two lots.

EXPERIMENT 25.

In this experiment it was decided to attempt to ascertain the degree of immunity established by subcutaneous and intramuscular injections on the same lines as adopted for the sheep experiments. As no reliable minimal lethal dose of virulent spores could be determined for horses, the standard for sheep was adopted.

A. Subcutaneous injection of the same vaccine as used above.

Horse.	Date Vaccine, 3/8/27.	Result.	Date Virulent Spores.		Result.
	c.c. subcut.			M.L.D.	
17967.......	1	Negative.........	15/9/27	20,000	Alive.
18230.......	1	Small flat swelling	28/10/27	40,000	,,
18213.......	1	Slight nodular swelling	25/8/27	10,000	,,
17789.......	1	Slight swelling...	28/10/27	50,000	,,
18097.......	1	Negative.........	15/9/27	20,000	,,

Result.—Three out of the five horses vaccinated showed small swellings. All the animals withstood an immunity test as high as 50,000 M.L.D. (sheep standard).

B. Deep intramuscular preventing skin contamination.

Horse.	Date Vaccine, 3/8/27.	Result.	Date Virulent Spores.		Result.
	c.c.			M.L.D.	
17737.......	1	Negative.........	25/8/27	10,000	Alive.
18089.......	1	,, ...	28/10/27	40,000	,,
18079.......	1	,, ...	,,	40,000	,,
17860.......	1	Very slight swelling	,,	50,000	,,
18464.......	1	Very slight swelling	,,	50,000	,,

Thus, two out of the five horses showed very slight swellings after vaccination, while all withstood the immunity test, which in some cases was applied practically three months after vaccination.

Control.—Horse 18100 was injected with 10,000 M.L.D. (for sheep) of the virulent spore emulsion used above. The animal died from anthrax within five days. Horse 16621 was given by the mouth 100,000 M.L.D. (for sheep) of the same spore emulsion, without, however, showing any ill effect.

From the preceding experiments, as well as from a number of other tests conducted on horses, it seems very difficult to predict the development of swellings, since in many cases it may result from the use of a weak vaccine and not from a stronger one. Further, it frequently happens that a retest reveals totally different results. With regard to the different methods of vaccination, swellings have been noted with each, in practically the same proportion of cases, so that no definite advantages could be ascribed to any particular one method from this respect. Although it is difficult to ascertain within narrow limits the M.L.D. of virulent material for horses, there is little doubt that a strong immunity is developed, e.g. the vaccinated horses withstood as much as 50,000 sheep M.L.D. whereas one control died of 10,000 M.L.D. (sheep).

5. SUMMARY AND GENERAL DISCUSSION.

As mentioned before, the main object of this work was to ascertain:—(*a*) To what extent the metabolic products elaborated by the Bacillus anthracis either in vitro or in the animal body could be utilized as active immunizing agents against the disease. (*b*) To compare the degree of immunity developed by different methods of vaccination.

In the foregoing report an account was given of the various experiments conducted and the results that were obtained. It is now intended to summarize and discuss these results.

1. *Introduction.*—Mention was made of the great importance of the anthrax problem in most countries of the world, and as a result of it the tremendous amount of research work carried out. Although much valuable knowledge had been gained in combating the disease, numerous points in connection with the biology of the organism both in nature and in the animal body were still unsolved.

Practically every make of anthrax vaccine used is composed of the living but attenuated organism either in the vegetative or in spore form. Vaccine prepared from a well-selected strain and carefully prepared, as a general rule affords a good active immunity in most of the domestic animals, and is indeed, together with the proper disposal of anthrax carcases, our only means of eradicating the disease. There seems to be strong evidence that once a particular strain had been attenuated to the vaccine stage, subsequent increase in virulence does not take place, although animals may occasionally die from the attenuated strain following vaccination. A more ideal vaccine, however, would be one from which the living organism has been excluded.

2. *Selection of Experimental Animals.*—Before conducting immunity work on anthrax, as on any other disease, it is essential that the experimental animals are carefully selected, especially where there are marked differences in susceptibility and immunity exhibited by the various species to the disease in question. Failure to appreciate this fact may easily lead to erroneous conclusions. The small laboratory animals, although markedly susceptible to anthrax, are difficult to immunize, hence their unreliability for such work. Horses and cattle on the other hand, although susceptible to natural infection with anthrax, are difficult to kill by artificial infection. Outbreaks of anthrax among these animals may be stopped by vaccination, clearly showing that immunity is developed, although it is difficult to ascertain the degree of immunity, since many animals show an inherent resistance to artificial infection.

The ideal species of animal for the work is one showing a practically uniform susceptibility to artificial infection combined with the quality of developing after vaccination an active immunity, and which can be fairly accurately tested within a suitable period by the administration of virulent material. From the various experiments conducted it would seem that merino sheep, and to a lesser extent the ordinary domesticated goat, are more suitable for this work than any of the other species tried out. It is for this reason that practically all the immunity experiments were conducted on sheep and in some cases also on goats.

3. *Attenuation of Virulent Strains.*—Several strains of virulent anthrax were attenuated by the Pasteur method, i.e. the prolonged incubation of broth cultures at a temperature of 42° C. Subcultures on to nutrient agar were made every five days, and test batches of spore vaccine prepared from different attenuations and also from different colonies of the same attenuation. The virulence of the test vaccines was ascertained by injecting small animals. From the results obtained, it was clear that some strains took much longer to become attenuated than others, while even in the same strain there was an unequal attenuation of the organisms, some showing marked

virulence even after 80 days' incubation at 42° C. Although the vaccines prepared from many of the attenuations proved safe, the immunizing power in many instances was unsatisfactory, as shown by tests on sheep and goats. The only reliable method of obtaining an efficient vaccine strain seems to be the preparation and testing out of numbers of test batches of vaccine prepared from the different attenuations, and even from different colonies, of several strains. It would appear that there exists a marked individuality of strains, and perhaps even of the organisms in the same strain.

Attenuation was also attempted by cultivating virulent organisms for long periods in various uncommon liquid media. From the small animal tests it would appear, however, that attenuation was in no way hastened by this method. The repeated alternate freezing and thawing of young virulent anthrax bacilli was not found to cause any decrease in virulence for guineapigs, rabbits and white mice, and hence proved unsuitable for the production of a vaccine strain. The Pesteur method, although frequently very tedious and disappointing, seems to be the best method of attenuating anthrax strains.

4. *Disintegrated Anthrax Cultures.*—Saline emulsions prepared from the surface growth of young anthrax cultures were disintegrated either by physical or chemical means. Sterile germ-free filtrates were obtained by passing this material through bacterial filters. Such filtrates when injected into animals were neither toxic to guineapigs and white mice, nor did they provoke immunity in sheep and goats against the disease. These experiments tend to exclude the presence of any endocellular toxins or immunizing substances within the anthrax bacilli cultivated on artificial media and subsequently disintegrated.

5. *Washed Anthrax Cultures.*—Vaccine spores as well as young vegetative forms derived from these spores were thoroughly washed free from all extracellular substances by repeated shaking up in saline and centrifugalizing. Both the washed spores and the washed bacilli when injected into guineapigs and mice, produced death from anthrax the same as did the unwashed material. In this respect B. anthracis differs markedly from the B. chauveaui, where the presence of its metabolic products is essential in causing the death of an animal . Sheep and goats developed a solid active immunity when vaccinated either with the washed anthrax spores or the washed bacilli.

6. *Metabolic products elaborated by the Bacillus anthracis in vitro.*—Both virulent and vaccine strains of anthrax were cultivated in 15 different liquid media for periods up to five weeks, after which time the material was passed through bacterial filters and a sterile filtrate obtained. The object of these experiments was to ascertain the toxic or immunizing properties of such filtrates. Varying amounts of each filtrate were injected into guineapigs and mice, without producing any ill effect on any of the animals. This excludes the presence of any extracellular toxins elaborated in these different media. When tested out on sheep and goats, these filtrates were completely safe, but of no immunizing value, since with one or two exceptions all the animals died from anthrax after the immunity test. In two sheep injected with filtrate prepared by growing B.

anthracis in ascites fluid from a dog mixed with an equal quantity of ordinary nutrient broth, the immunity test was withstood. It would thus appear that no metabolic products comparable to the "aggressin" of the B. chauveaui or to the toxin of B. tetani were elaborated by the B. anthracis in artificial media. Such products, if present, were neither toxic nor of any real immunizing value.

7. *Metabolic products elaborated by the Bacillus anthracis in the animal body.*—An imported product, so-called "true anthrax aggressin," prepared from the juices extracted from anthrax carcases, was tested out on sheep and goats. Although completely safe, its immunizing value was negligible, since all eight animals vaccinated with it died from anthrax after the immunity test. In order to ascertain the presence of any such substances, susceptible sheep and goats were infected with virulent material. From these animals, blood was collected shortly before death when numerous bacilli had appeared in the peripheral circulation. Peritoneal fluid and bile, as well as certain organs for the preparation of extracts, were collected after death. Sterile germ free filtrates prepared from some of the diluted blood, peritoneal fluid, bile and organ extracts, were injected into small animals without producing any toxic effects. The same products when tested out on sheep and goats showed practically no immunizing value since out of a toal of 14 sheep, 12 died from anthrax after the immunity test. Instead of filtering, some of the blood samples mentioned above were treated with (*a*) high dilutions of neosalvarsan, (*b*) with anilin gentian violet immediately afte1 the blood was drawn. (Previously conducted experiments had shown that these substances had a marked bactericidal effect on the B. anthracis.) Subcultures prepared from this blood 24-48 hours after showed it to be sterile, while on microscopic examination the bacilli showed marked disintegration. Guineapigs and mice injected with this material showed no ill effect. Sheep and goats were then injected with 2 c.c. and 5 c.c. amounts and tested with virulent spores three to four weeks afterwards. As all the animals remained alive, immunity had undoubtedly been produced by the disintregrated blood. After five months standing, this blood was again tested on sheep and goats. In order to ascertain the thermostability some of the blood was pasteurized as high as 92° C. for 30 minutes. The immunity produced was in no way inferior to that noted in the previous test. Out of a total of 20 sheep and goats vaccinated with the disintegrated anthrax blood 18 withstood an immunity test ranging from 100 to 250 M.L.D. Further, this immunizing substance was still potent after a period of five months and was not destroyed by heating to 92° C. for 30 minutes. These results were directly opposed to those obtained with the filtered blood and other body juices where 12 out of the 14 sheep died after the immunity test. The only explanation for these conflicting results would seem to be that the immunizing substance, undoubtedly present in the blood, was not filterable, i.e. it is not of the nature of a soluble filterable aggressin as found in blackquarter, but that it is closely associated with the organisms present in the blood, probably either in the bacterial body itself or in the capsular material surrounding it. It thus seems probable that the bacillary debris present in the blood contains in itself an active and reliable immunizing agent. As mentioned before,

this could not be established for artificially cultivated organisms which when disintegrated, either by physical or chemical means, or even killed by pasteurization, did not exhibit any immunizing powers. In this respect the anthrax bacillus, found in the circulating blood, when compared with the organism cultivated on artificial media, seems to possess totally different qualities.

It should be mentioned that of all the inert non-living anthrax products obtained, either from artificial cultivation or from the animal body, or from any other sources, this disintegrated unfiltered blood was the only material which produced a definite and reliable active immunity. The fact that in practice more or less all the anthrax vaccines in use consist of the attenuated living organisms shows that " dead " or sterile vaccine does not produce the same strong immunity.

The oedema fluid collected from horses showing swellings after vaccination was not found to be toxic for small animals, nor did it produce swellings in other horses. Its immunizing value, as tested out on sheep and goats, was negligible.

8. *Comparative Safety and Immunity Experiments with Anthrax Spore Vaccine.*—Under this heading an account is given of a number of different experiments. In the first series of experiments an attempt was made to ascertain the susceptibility of guineapigs to different routes of infection. For this purpose a fairly weak spore vaccine was used. Except where cuti-vaccination was deliberately intended, careful precautions were taken to prevent as far as possible, contamination of the skin with vaccine; e.g. on subcutaneous or intramuscular injections the needle wound was immediately disinfected by being swabbed out with a fine pinect, the points of which were rolled in cotton wool and steeped in 10 per cent. tincture of iodine. By this procedure it was found that these animals readily succumbed from anthrax after subcutaneous, intraperitoneal and intramsucular injections, while with intradermal injection death was uncertain. By oral and intratracheal administration, none of the animals were killed.

In order to ascertain if any immunity was developed by different methods of inoculation, a number of guineapigs were injected with smaller amounts of the same vaccine that had killed before. Most of the animals survived, and after two-three weeks the immunity was tested by injecting small amounts of a stronger vaccine. Those vaccinated intraperitoneally, subcutaneously, intratracheally and orally all died after the immunity test, showing that no immunity had been developed. It seemed fairly definite, however, that some vaccinated deep intramuscularly had developed immunity since those not killed by the vaccine all withstood the immunity test. The result of the intradermal vaccination was doubtful since only two out of the five withstood the immunity test. It thus seemed clear that guineapigs showed well-marked susceptibility to most of the routes of infection, except to oral and intratracheal administration, while in only those vaccinated deep intratracheal was the development of immunity fairly definite.

Using the same weak vaccine several experiments were conducted on sheep for the purpose of comparing the degree of immunity conferred by subcutaneous, intramuscular, and intradermal vaccination respectively. Contamination of the skin was prevented, except in intradermal vaccination. After two-three weeks the immunity was tested with virulent spores in amounts ranging from 250 to 3,000 M.L.D. Of the 12 sheep vaccinated *subcutaneously*, seven died from anthrax after the immunity test. Of the 12 sheep vaccinated *intradermally*, seven died from anthrax after the immunity test, while of the 12 vaccinated *deep intramuscularly*, only one died after the immunity test. These results were obtained from the three separate experiments which were carried out. It thus seems definite that where a weak spore vaccine is used the immunity established by intramuscular inoculation is much stronger than that given by either subcutaneous or intradermal vaccination. No difference was noted in the degree of immunity developed by the latter two methods.

This experiment was repeated on goats, using the same weak vaccine. The results confirmed those obtained with sheep. Three out of the five goats vaccinated subcutaneously died after the immunity test, while all five vaccinated intramuscularly survived the test. In a subsequent experiment where a strong cattle vaccine was used on goats, five out of ten goats died from vaccination, while the surviving five all withstood an immunity test of 1,500 M.L.D. Of ten goats vaccinated deep intramuscularly, three died from the result of vaccination, while the surviving seven all withstood an immunity test of 1,500 M.L.D. This clearly shows the susceptibility of goats to a strong vaccine. As all the goats which survived the vaccination also survived the immunity test, it would appear that where a strong vaccine is used the difference in the degree of immunity following (*a*) subcutaneous and (*b*) intramuscular vaccination, is not so striking as when a weak vaccine is used. The immunity may even be the same with the two methods where the strong vaccine is used.

A number of experiments were then conducted on horses, the main object being to ascertain what factors were responsible for the development of the oedematous swellings so frequently noted in this species after vaccination. Subcutaneous injections were made with sterile broth filtrate in which anthrax had been cultivated for long periods. In none of the animals were swellings noticed. Oedema fluid, collected from horses showing such swellings, when injected into other horses also gave negative results. Washed vaccine spores, freed from extracellular material, produced swellings in some cases, while in others no swellings were noticed. By subcutaneous, intramuscular and intradermal vaccination, using a glycerinized spore emulsion, swellings were obtained in some cases and not in others.

Further, it was noticed that whereas marked swellings followed vaccination in a troop of horses, a retest of the same vaccine on a different lot of horses frequently resulted in none or only a few of the animals showing swellings. Certain experiments which were conducted also clearly showed that while a definitely weak vaccine may cause swellings in horses, a much stronger vaccine, as e.g., used for cattle and sheep, may be completely safe for horses, i.e. no swellings are developed. Thus the strength of the vaccine itself cannot be considered as the sole factor determining the development of swellings; neither could the method of vaccination be the cause, since swellings were noted in practically equal proportions following different methods of vaccination. Since anthrax broth filtrates, as well as oedema fluid from other horses, did not cause swellings, it would seem that extracellular toxins cannot be incriminated as being the cause.

The possibility of idiosyncrasy of animals, combined perhaps with certain environmental conditions, may account for these inconsistent results.

6. CONCLUSIONS.

From experiments carried out the following conclusions would appear to be justified:—

1. The small laboratory animals, although markedly susceptible to anthrax, are difficult to immunize, hence their unreliability in a study on anthrax immunity. Horses and cattle on the other hand, although susceptible to natural infection with anthrax, are difficult to kill by artificial infection. The merino sheep, which shows a practically uniform susceptibility to artifical infection, combined with the quality of developing an active immunity after vaccination, seems to be an ideal species for immunity work on anthrax.

2. Attenuation of different anthrax strains, according to the Pasteur method, shows that some strains take much longer to become attenuated than others, while even in the same strain there is unequal attenuation of the organisms, some showing marked virulence even after 80 days' incubation at 42° C. Thus there appears to be a marked individuality of strains and even of single organisms in the same strain.

3. Cultivation of B. anthracis in various uncommon liquid media does not hasten attenuation. Similarly repeated alternate freezing and thawing of anthrax cultures fails to cause any decrease in virulence.

4. By physical or chemical disintegration of artificially cultivated anthrax organisms the sterile cell substance is found to be free from both toxic and immunizing properties.

5. Anthrax spores, as well as the vegetative forms, when washed free from all extracellular material are by themselves capable of either causing a fatal infection or of producing an active immunity.

6. Prolonged cultivation of both virulent and vaccine anthrax strains in different uncommon liquid media, followed by subsequent filtration, fails to reveal either toxic or immunizing properties in the filtrate.

7. Blood drawn at the time of death from animals infected with virulent anthrax, and sterilized by chemical means, is seen to possess well-marked immunizing properties. This quality is maintained in blood kept for five months and resists heating at 92° C. for 30 minutes. Such blood produces an active immunity. Filtrate obtained from freshly drawn anthrax blood fails to reveal any such immunizing powers. Thus the unfilterable bacillary debris itself seems to be the immunizing substance in the blood. The debris obtained by disintegrating artificially cultivated organisms does not show the same antigenic properties.

8. By utilizing anthrax spore vaccine on guineapigs, lethal infections can be produced by subcutaneous, intraperitoneal or intramuscular injections, while the results of intradermal application are uncertain. With oral or intratracheal administration these animals are not easily killed.

9. The nature and degree of immunity developed in sheep following *intradermal* vaccination with anthrax spore vaccine is no stronger nor more permanent than that following *subcutaneous* inoculation. Following the use of fairly weak anthrax spore vaccine, the immunity established by *intramuscular* vaccination seems to be definitely superior to that produced by either subcutaneous or intradermal injections. With a strong vaccine these differences are less marked.

10. In horses the strength of the vaccine cannot be considered as the sole factor determining the development of swellings, since weak vaccines may provoke swelling, whereas stronger ones may fail to do so. Further, the method of vaccination cannot be the responsible cause, since approximately the same percentage of animals show swellings after intradermal, subcutaneous or intramuscular vaccination.

Lastly, anthrax broth filtrates as well as oedema fluid from other horses showing anthrax swelling do not provoke swellings in susceptible horses, and hence extracellular toxins cannot be incriminated as being the cause.

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The Relation of the Virulence of Attenuated Anthrax Strains to their Immunizing Value.

By J. G. BEKKER, B.V.Sc., Veterinary Research Officer, Onderstepoort.

ONE of the first essentials for the production of good Anthrax spore vaccine is a suitable strain. By a good vaccine is understood one that can be used with comparative safety in a particular species of animal, and which at the same time provokes a high degree of immunity.

Data have here been collected from the virulence and immunizing tests to which various strains of attenuated anthrax have been subjected in the Anthrax Department of the Veterinary Research Laboratories at Onderstepoort. Before a strain is selected for vaccine purposes a small test batch is prepared from it, and if the test batch proves successful a large routine batch is then made. After a very thorough test, this vaccine is issued for use in the field.

Viljoen, Curson, and Fourie in their report, "Anthrax in South Africa." 13*th and* 14*th Report of the Director of Veterinary Education and Research*, describe in full detail the methods employed in the production of this spore vaccine, and it is unnecessary to describe them again. The same methods were employed throughout in the course of the production of the various trial batches found in this report.

It will become apparent from the data offered here that when anthrax spore vaccine is made, two distinct biological characteristics must be recognized, viz.:—

(1) The virulence of the strain of attenuated organism used. and

(2) The quality which the organism has of stimulating the production of immune bodies in an animal. This property is generally described as the immunizing value.

In textbooks where the production of anthrax vaccine is described, it appears a very simple matter. Generally the importance of the immunizing value of a particular strain is not even mentioned, and the impression is obtained that if any strain of anthrax is sufficiently attenuated it will suffice for vaccine purposes.

A considerable variation in the immunizing value in different strains has, however, been noted in the course of work on anthrax in this Laboratory. Some of the strains were collected from field specimens submitted from various parts of South Africa. They were suitably attenuated according to the method described by Viljoen, Curson, and Fourie. Other strains were collected from imported vaccines, and test batches prepared from them.

The same technique was used for preparing the various batches included in this report, and it will be observed that more or less the same amount of vaccine was administered to the test animals. For the virulent test the same virulent material was used throughout. The dose of vaccine cannot, of course, be absolutely controlled. A more or less constant amount of normal saline was used for washing off the spores in the Fernbach flasks; but normal errors of calculation, etc., are liable to occur. For this reason the doses of vaccine administered are not absolutely identical, although that does not greatly affect the ultimate results obtained.

The results of the following test batches are included:—

BATCH I.

Prepared: 15.6.25.
Strain: Winburg, O.F.S.
Attenuation: For 60 days at 42.5° C.
Sporulation: Very good.

Small Animal Safety Test: Animals injected 24.6.25.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c.................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax. 28/6/25.
1 Guinea-pig..............	0·01 ,, ...	† ,, 2/7/25.
1 Guinea-pig..............	0·001 ,, ...	† ,, 29/7/25.

Large Animal Safety and Immunizing Test.

Animal No.	Amount of Vaccine. Date 18/8/25.	Result.	Virus. Date 8/9/25.	Result.
Sheep 10457	0·02 c.c.....	Lived...........	500 M.L.D. B.5...	†Anthrax. 12/9/25
11071	0·02 ,, ...	,, ...	500 ,, ...	† ,, 14/9/25
9125	0·01 ,, ...	,, ...	500 ,, ...	Lived.
10552	0·01 ,, ...	†Anthrax. 27/8/25		
Goat 11876	0·02 ,, ...	Lived...........	250 ,, ...	†Anthrax. 11/9/25
11872	0·01 ,, ...	†Anthrax, 2/9/25		

BATCH II.

Prepared: 9.6.25.
Strain: Hoopstad, O.F.S.
Attenuation: 60 days at 42.5° C.
Sporulation: Good.

Small Animal Safety Test: Animals injected 24.6.25

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c..................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax, 26/6/25.
1 Guinea-pig..............	0·01 ,, ...	† ,, 26/6/25.
1 Guinea-pig..............	0·001 ,, ...	† ,, 27/6/25.

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 6/7/25.	Result.	Virus. Date 27/7/25.	Result.
Sheep 8444	0·02 c.c. ..	Lived...........	500 M.L.D. B.5 ..	†Anthrax, 29/7/25
11115	0·02 ,, ..	,, ...	500 ,, ..	† ,, 30/7/25
10252	0·01 ,, ..	,, ...	500 ,, ..	Lived.
11343	0·01 ,, ..	†Anthrax, 23/7/25		
Goat 11844	0·02 ,, ..	Lived...........	250 ,, ..	†Anthrax, 30/7/25
11867	0·01 ,, ..	,, ...	250 ,, ..	† ,, 29/7/25

BATCH III.

Prepared: 12.11.25.
Strain: Boshof, O.F.S.
Attenuation: 80 days at 42.5° C.
Sporulation: Very good.

Small Animal Safety Test: Animals injected 20.11.25.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c..................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax, 25/11/25.
1 ,, ,, ...	0·01 ,, ...	† ,, 23/11/25.
1 ,, ,, ...	0·001 ,, ...	Lived.

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 2/12/25.	Result.	Virus. Date 18/12/25.	Result.
Sheep 9005	0·02 c.c.....	Lived...........	500 M.L.D. B.5...	Lived.
8865	0·01 ,, ...	,, ...	500 ,, ..	,,
Goat 14031	0·02 ,, ...	,, ...	250 ,, ..	,,
14024	0·01 ,, ...	,, ...	250 ,, ..	,,

BATCH IV.

Prepared: 22.12.27.
Strain: No. 42. (From imported cattle vaccine.)
1st of double vaccine.
Sporulation: Excellent.

Small Animal Safety Test: Animals injected 4.1.28.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c..................	Lived.
1 Guinea-pig..............	0·1 „	†Anthrax, 7/1/28.
1 „ „	0·01 „	† „ 10/1/28
1 „ „	0·001 „	† „ 9/1/28

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 24/1/28.	Result.	Virus. Date 13/2/28.	Result.
Sheep 17124	0·02 c.c.....	Lived...........	1,000 M.L.D. B.5.	Lived.
17742	0·01 „	„	1,000 „	„
17022	0·01 „	„	1,000 „	„
Goat 18602	0·02 „	„	500 „	„
18611	0·01 „	„	500 „	„

BATCH V.

Prepared: 22.12.27
Strain: No. 43. (Isolated from imported cattle vaccine.)
2nd of double vaccine.
Sporulation: Excellent.

Small Animal Safety Test: Animals injected 4.1.28.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c..................	Lived.
1 Guinea-pig..............	0·1 „	†Anthrax, 6/1/28.
1 „ „	0·01 „	† „ 7/1/28
1 „ „	0·001 „	† „ 13/1/28.

Large Animal Safety and Immunity Test.

Animal No.	Vaccine. Date 24/1/28.	Result.	Virus. Date 13/2/28.	Result.
Sheep 16541	0·02 c.c....	Lived..........	1,000 M.L.D. B.5.	†Anthrax, 22/7/28
17481	0·01 ,, ...	†Anthrax, 2/2/28		
11627	0·01 ,, ...	Lived..........	1,000 ,,	Lived.
Goat 18630	0·02 ,, ...	,, ...	500 ,,	,,
18634	0·01 ,, ...	,, ...	500 ,,	,,

BATCH VI.

Prepared: 22.12.27
Strain: No. 44. (From imported cattle vaccine. Single.)
Sporulation: Very good.

Small Animal Safety Test: Animals injected 4.1.28.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c................	Lived.
1 Guinea-pig.............	0·1 ,, ...	†Anthrax, 7/1/28.
1 ,, ,, ...	0·01 ,, ...	† ,, 8/1/28.
1 ,, ,, ...	0·001 ,, ...	† ,, 9/1/28.

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 24/1/28.	Result.	Virus. Date 13/2/28.	Result.
Sheep 16622	0·02 c.c....	Lived..........	1,000 M.L.D. B.5.	Lived.
11625	0·01 ,, ...	,, ...	1,000 ,,	,,
17553	0·01 ,, ...	,, ...	1,000 ,,	,,
Goat 18631	0·02 ,, ...	,, ...	500 ,,	,,
18624	0.01 ,, ...	†Anthrax, 1/2/28		

BATCH VII.

Prepared: 22.12.27.
Strain: No. 45. (From imported cattle vaccine. Single.)
Sporulation: Good.

Small Animal Safety Test: Animals injected 4.1.28.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c.................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax, 8/1/25.
1 ,, ,, ...	0·01 ,, ...	† ,, 10/1/25.
1 ,, ,, ...	0·001 ,, ...	†Lived.

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 24/1/28.	Result.	Virus. Date 13/2/28.	Result.
Sheep 9123	0·02 c.c.....	Lived...........	1,000 M.L.D. B.5.	†Anthrax, 17/2/28
17670	0·01 ,, ...	,, ...	1,000 ,,	† ,, 17/2/28
17529	0·01 ,, ...	,, ...	1,000 ,,	† ,, 16/2/28
Goat 18613	0·02 ,, ...	,, ...	500 ,,	† ,, 17/2/28
18380	0·01 ,, ...	,, ...	500 ,,	Lived.

BATCH VIII.

Prepared: 22.12.27.
Strain: No. 48. (From imported cattle vaccine. Single.)
Sporulation: Very good.

Small Animal Safety Test: Animals injected 4.1.28.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c.................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax, 8/1/28.
1 ,, ,, ...	0·01 ,, ...	† ,, 8/1/28.
1 ,, ,, ...	0·001 ,, ...	† ,, 9/1/28.

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 24/1/28.	Result.	Virus. Date.	Result.
Sheep 17681	0·02 c.c.....	Lived...........	1,000 M.L.D. B.5	Lived.
17686	0·01 ,, ...	,, ...	1,000 ,,	,,
13708	0·01 ,, ...	,, ...	1,000 ,,	,,
Goat 18593	0·02 ,, ...	,, ...	500 ,,	,,
18599	0·01 ,, ...	,, ...	500 ,,	,,

BATCH IX.

Prepared: 11.11.27.
Strain: Rustenburg.
Attenuation: 60 days at 42.5° C.
Sporulation: Excellent.

Small Animal Safety Test: Animals injected 18.11.27.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c..................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax, 25/11/27.
1 ,, ,, ...	0·01 ,, ...	† ,, 26/11/27.
1 ,, ,, ...	0·001 ,, ...	† ,, 26/11/27.

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date 5/12/27.	Result.	Virus. Date.	Result.
Sheep 17399	0·02 c.c.....	Lived..........	1,000 M.L.D. B.5.	†Anthrax, 1/1/28
17283	0·02 ,, ...	†Anthrax, 9/12/27		
17524	0·01 ,, ...	Lived..........	1,000 ,,	Lived.
17719	0·01 ,, ...	,, ...	1,000 ,,	†Anthrax, 2/1/28
Goat 18641	0·02 ,, ...	†Anthrax, 9/12/27		
18605	0·1 ,, ...	Lived..........	500 ,,	Lived.

BATCH X.

Prepared: 22.12.27
Strain: No. 49. (From imported cattle vaccine. Single.)
Sporulation: Not good.

Small Animal Safety Test: Animals injected 4.1.28.

Animal.	Amount of Vaccine.	Result.
1 Rabbit..................	0·1 c.c..................	Lived.
1 Guinea-pig..............	0·1 ,, ...	†Anthrax, 8/1/28.
1 ,, ,, ...	0·01 ,, ...	Lived.
1 ,, ,, ...	0·001 ,, ...	,,

Large Animal Safety and Immunity Test.

Animal No.	Amount of Vaccine. Date.	Result.	Virus. Date 13/2/28.	Result.
Sheep 17477	0·02 c.c.....	Lived...........	1,000 M.L.D. B.5.	†Anthrax, 16/2/28
17141	0·01 ,, ...	,, ...	1,000 ,,	Lived.
17722	0·01 ,, ...	,, ...	1,000 ,,	†Anthrax, 16/2/28
Goat 18615	0·02 ,, ...	,, ...	500 ,,	Lived.
18614	0·01 ,, ...	,, ...	500 ,,	†Anthrax, 17/2/28

It will be seen that Batches I and II prepared from two different strains and attenuated for 60 days at 42.5° C. had practically the same virulence. In each case all the guinea-pigs were killed. In Batch I, Sheep 10552 and Goat 11872 were also killed by the vaccine. Batch II killed Sheep 11343. Batch III, on the other hand, was prepared from a strain that was attenuated in the same way for 80 days. As can be seen in the subsequent tests, its virulence was certainly less, as only two guinea-pigs were killed and none of the larger animals. The immunity conferred by Batches I and II was very unsatisfactory, while Batch III immunized well. The immunizing value of these particular strains does, therefore, not seem to depend upon their virulence.

By studying the results obtained in Batches IV, V, VI, VII, VIII, IX, and X the same conclusion can be drawn. These batches were prepared from the various strains as indicated. Six out of the seven were made from strains isolated from imported vaccines. Batch No. IX was perpared from a locally obtained strain. They are grouped together, as the virulence test in each case is the same. The source of the vaccine from which the strains were isolated is not mentioned, for obvious reasons. These strains (Nos. 42, 43, 44, 45, 48, and 49) were all prescribed for use in sheep and cattle.

The results obtained from these batches may be summarized as follows:—

Batch No.	The Virulence as indicated by the Animal Killed.	The Immunity conferred. (The Result of Virulent Test.)
IV........	All guinea-pigs killed...............	*Good :* All animals lived.
V........	All guinea-pigs killed............... Sheep (17481) killed.	*Fair :* All animals, except sheep 16541, lived.
VI........	All guinea-pigs killed.............. 1 Goat (18624) killed	*Good :* All animals lived.
VII........	2 out of 3 guinea-pigs killed........	*Very Bad :* 3 sheep (9123, 17670, 17529); 1 goat (18613) died.
VIII........	All guinea-pigs killed...............	*Very Good :* All animals lived.
IX........	All guinea-pigs, 1 sheep (17283), and 1 goat (18641) killed	*Bad :* Two sheep (17399, 17719) died.
X........	1 out of 3 guinea-pigs killed........ 1 goat (18624) killed	*Bad :* Two sheep (17477, 17722); one goat (18614) died.

Batch IV prepared from the 1st of a double vaccine proved to be better in its immunizing value than Batch V prepared from 2nd of double.

Batch IX was the most virulent, yet it conferred the least degree of immunity.

Batch VII, although not so virulent, was equally useless for immunizing.

Batch X should really not be included here as its virulence is decidedly weaker than that of the other batches. It is mentioned, however, to show that this strain, isolated from a vaccine which was issued for use in cattle and sheep, is, comparatively speaking, useless.

In all these batches accurate note was made of the degree of sporulation. Only in Batch X could the sporulation be described as weak. The sporulation ranged from good to excellent in other batches. It does not seem as if the actual degree of spore formation in these particular tests has any demonstrable effect on the immunizing qualities. The spore formation is, however, of great importance when considering the keeping qualities of a vaccine.

With the present extent of our knowledge it is impossible to state the reason why anthrax strains, when attenuated, should vary in their immunizing qualities. When a better explanation of the nature of immunity in anthrax is forthcoming, it might be possible to explain this phenomenon.

It has been claimed by some workers that immunizing bodies are present in the tissues and fluids of animals which have succumbed to the disease. The immunity produced by these bodies does not seem to be great, in any case not as strong and lasting as that produced by the living organism (spores or bacilli), even in its much attenuated form.

When the mechanism of anthrax immunity becomes evident, the variation in the immunizing value of anthrax strains will probably be explained. Some strains may have an inherent quality of producing a greater amount of the substance which stimulates the animal cell to produce immune bodies.

South African prepared vaccine has given very good results. It is used extensively. The good results obtained with this vaccine can no doubt be partly put down to the very careful selection of strains of attenuated anthrax bacilli used in producing these vaccines.

Conclusions.

1. The immunizing value of an anthrax strain does not depend entirely upon the degree of attenuation to which it has been subjected.
2. A marked variation in immunizing value exists in different strains.

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Immunization against Anaerobes of the Gas-Gangrene Type in South Africa by means of "Anatoxins."

By W. J. B. GREEN, B.V.Sc., Veterinary Research Officer,
Onderstepoort.

INTRODUCTION.

THE treatment of certain bacterial antigens or toxins with formaldehyde renders them inert, without, however, affecting their antigenic powers. To these modified toxins which have lost their toxicity, but retained their immunizing power, the term "anatoxins" was first applied by Ramon. Ramon, however, was not the first to notice this peculiar and remarkable action of formalin, as, from a study of the literature, it appears that as early as 1904 diphtheria toxin modified by formalin was used by Glenny and Hopkins in the immunization of horses for antitoxin on a large scale.

As an example of the action of formalin on toxin, the work of Löwenstein, 1909, on Tetanus may be quoted. He described a tetanus toxin the M.L.D. of which was 1:10,000 for mice. This toxin after being treated with formol and left standing at room temperature for a long period, lost its toxicity for the greater part, as 5 c.c. could be injected subcutaneously into mice without harm. This modified toxin, "toxoid" or "atoxic toxin" as he called it, however, retained its immunizing property, as mice inoculated with 5 c.c. of this "atoxic toxin" were able to withstand 50,000 M.L.D.'s of fresh tetanus toxin.

Costa, Boyer, and Placidi successfully immunized rabbits against the bacillus of swine erysipelas by means of broth cultures killed with formaldehyde. According to them the method was used as early as 1920 by Costa.

The invaluable work of G. Ramon from 1922 onwards on the diphtheria anatoxin opened up a new field for experimental research. He found that if .3 to .4 per cent. formol was added to the toxin and kept at 40° C., a very active toxin was detoxicated after one month, and 10 c.c. of the anatoxin could be injected into a guinea-pig without any harmful result, whereas 1/800 c.c. of the unmodified toxin killed a guinea-pig. 1 c.c. of this anatoxin, injected subcutaneously, was sufficient to immunize against several M.L.D.'s 18 days later and against 50 to 100 M.L.D.'s after one month. Two injections of anatoxin at three weeks' interval protected 10 days after the second injection against 1,000 and more M.L.D.'s.

If injected into young horses, 1 c.c. or more of the anatoxin produced from 30 to 100 antitoxic units in the serum 8 to 10 days later. Following on these experiments, he showed that an active immunity could be established in man with the use of anatoxin, and determined an easy and practical method of inoculation. By means of the Floculation reaction which he describes, he was able to determine quickly and accurately the antigenic power of any particular anatoxin " in vitro." He further established the fact that, besides bacterial toxins, other more or less similar poisons, e.g. vegetable poisons like abrin and snake poisons like cobra venom, could be changed by the use of formalin into harmless substances which keep their antigenic properties.

Descombey, 1924, showed that everything that had been established in connection with diphtheria anatoxin applied to tetanus anatoxin as well, and that it had marked immunizing powers.

Durand, 1925, carried out experiments on the detoxication of the Shiga dysentery bacillus with formaldehyde and reported good results from its use. He was also able to detoxicate the gonococcus, and reported good results from its use in gonorrheal ophthalmia.

It was only comparatively recently that the action of formaldehyde was applied to the anaerobes of the gasgangrene group. Weinberg and Prévot, 1924, have shown that these toxins can be converted into anatoxins, and Weinberg and Goy (1924 and 1925) described the preparation of botulinus anatoxin. For the preparation of these anatoxins they describe the addition of varying quantities of 40 per cent. formaldehyde as follows: For *B. perfringens*, .1½ to .2 per cent.; for *V. septique*, .2 to .3 per cent.; for *B. oedematiens*, *B. histolyticus*, and *B. sporogenes*, .2 to .4 per cent; for botulinus anatoxin, 2 per cent. After the addition of the formaldehyde, they are incubated at 37° C., and by withdrawing small quantities at varying periods the toxicity can be tested by injecting guinea-pigs. The period can thus be ascertained when the toxin passes over to anatoxin. Weinberg, Prévot, and Goy applied the Floculation test of Ramon to the members of the gas-gangrene group, and obtained indifferent results, excepting when centrifugalized toxin was used, which contains a number of microbes in suspension. As in the case of diphtheria, they found that two injections of anatoxin are usually necessary to provide immunity. For the production of antiserum in horses Weinberg and Prévot used whole cultures sterilized by the addition of formol, the toxin thus not being separated by centrifugalization from the bacilli. For this formalinized culture, " the anatoxin totale of Ramon," they used the term " ana culture," which, they explain, is not grammatically correct, but is simple and explains the term without confusion.

Leclainche and Vallée, 1925, reported that young cultures of *B. chauvoei* and *V. septique* were sterilized by the addition of .2 to .4 per cent. formol and that the cultures were of greater vaule in immunization than filtrates. They were the first to use formaldehyde as a method in obtaining a sterile black-quarter vaccine.

Subsequently Basset, 1925, Cordier, 1926, and McEwen used formaldehyde in the preparation of black-quarter and *Vibrion septique* vaccines.

The primary aim of this paper is to show the use of formalinized anaerobic antigens in immunization against those anarobes of the gas-gangrene type which play a rôle in South Africa. A second chapter has been devoted to experiments in connection with the pathogenesis of black-quarter.

PART I.

I.—Immunization against anaerobes of the gas-gangrene type which occur in South Africa by means of formalinized antigens.

BLACK-QUARTER.

Black-quarter is prevalent in all parts of South Africa and heavy losses occur annually unless preventive inoculation is carried out regularly. Up to the present vaccination has been carried out with black-quarter filtrate or the so-called artificial aggressin prepared according to the method of Nitta and improved later in South Africa by Sheppard, Viljoen, and Scheuber. This improved filtrate (see Reports No. 11 and 12 of the Director of Veterinary Education and Research) proved to be remarkably efficient in both laboratory and field tests, although the method of preparation is rather a lengthy and involved process. The method employed in obtaining a sterile black-quarter vaccine by the use of formaldehyde is simple, short, and highly efficient. For use on a large scale, large quantities can be prepared within a short period.

METHOD OF PREPARATION OF BLACK-QUARTER FORMALINIZED VACCINE.

Preparation of Media.—The preparation of the media is the same, with the exception of a few alterations, as that for the filtrate, fully described in the 11th and 12th Reports of the Director of Veterinary Education and Research, Part 1, page 499.

Large flasks of 10 to 25 litres capacity are employed. The constituents of the media are in proportion to the amount required, as follows:—

Distilled water, 1,000 c.c.
Minced liver (ox), 500 gm.
Sodium phosphate, .2 per cent.
Peptone, 1 per cent.
Sodium chloride, .3 per cent.

The flasks containing the media are sterilized in an autoclave at 110° to 115° C. for an hour on each of three successive days, and the P^h adjusted to 8.4 after sterilization. 1 per cent. Glucose is then added to each flask. The glucose in watery solution must be sterilized separately in the autoclave at 116° C. for half an hour on each of two successive days.

Inoculation.—The flasks are inoculated with Pasteur pipettes of 5-10 c.c. capacity, the seed material being obtained from 24-48 hour cultures grown in V. *hibler* brain medium.

Incubation and Growth.—The flasks are placed in a large incubator at 37° C. to 40° C. Within 24 hours a rapid growth with profuse gas formation is noted, and the flasks are allowed to remain in the incubator until all gas formation has subsided; this usually occurs in two to three days. The flasks are removed from the incubator, vigorously shaken up, and then left to stand for half an hour, which allows the large liver particles to settle to the bottom of the flask. The supernatent liquid containing the bacteria is siphoned off into large 40-litre flasks by means of a glass and rubber siphon and .4 per cent. formaldehyde of a 40 per cent. solution added. The flasks are placed in the incubator at 37° to 40° C., and after seven days the vaccine is ready for use. No incubation is really necessary, as the vaccine prepared in this way is sterile within 48 hours, because the formaldehyde kills the bacilli and spores within 48 hours, and there is no toxin present. No toxic action of the filtrate which has passed through the Seitz filter has ever been noted, and up to 500 c.c. of the filtrate has been injected subcutaneously and intraperitoneally without any harmful result being noted. The formalinized culture in the case of black-quarter can be termed "anaculture" as used by Weinberg. Incubation for one week or longer seems to improve the value of the vaccine as will be shown later in the paper.

Before the vaccine is issued for use, a routine test of each batch is carried out by subcutaneous inoculation in sheep and testing for immunity two to three weeks later.

Immunity Tests with Black-quarter Formalinized Vaccine.

As the black-quarter filtrate prepared according to the method of Viljoen and Scheuber had always proved itself remarkably efficient in the preventive inoculation, it was decided to give the newer method an extensive trial to prove its superiority or otherwise over the older method. Accordingly several batches of vaccine were prepared at different periods, as described above, and tested for efficacy.

In all the following experiments sheep were used, as experimentally they are the most susceptible animals we have, and more reliable and uniform results were obtained than with the use of guinea-pigs. The virus used in testing for immunity was prepared from v. Hibler's medium after 24 hours' growth, and in all cases injected intramuscularly into the left thigh on the inner surface. The minimum lethal dose in all the experiments is taken as the smallest dose of virus which kills the control sheep. The M.L.D. varies a little in some experiments, as the virulency of the culture is not always a constant factor. The intervals between the inoculation of vaccine and injection of virulent culture varied from 13 to 50 days.

EXPERIMENT No. 1. (S. 3066.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 1.

Sheep No.	Vaccine Subcutaneously, 10/5/27.	Virus Intramuscularly, 2/6/27.	Results.
15603	2 c.c.	1 c.c.	Lived.
15631	,,	2 ,,	,,
15652	,,	3 ,,	,,
15789	,,	4 ,,	,,
15803	,,	5 ,,	,,
15844	,,	6 ,,	,,
16632	,,	6 ,,	,,
16659	,,	8 ,,	,,
16706	,,	10 ,,	,,
16714	,,	12 ,,	,,
15610	Control	1 c.c.	Died, B.Q.
15612	,,	1·5 ,,	,,

The vaccine was tested 23 days after inoculation. All the sheep were immunized, the vaccine giving protection up to 12 minimum lethal doses.

EXPERIMENT No. 2. [S. 3221(A).]

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 2.

Sheep No.	Vaccine Subcutaneously, 27/8/27.	Virus Intramuscularly, 17/10/27.	Results.
16770	1 c.c.	2 c.c.	Lived.
16765	2 ,,	3 ,,	,,
16925	1 ,,	3 ,,	,,
17001	2 ,,	4 ,,	,,
17710	Control	2 c.c.	Died, B.Q.
17730	,,	2 ,,	,,

EXPERIMENT No. 3. (S. 3407.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 3.

Sheep No.	Vaccine Subcutaneously, 20/2/28.	Virus Intramuscularly, 10/4/28.	Results.
18903	1 c.c.	1 c.c.	Lived.
19035	1 ,,	1 ,,	,,
19042	2 ,,	1 ,,	,,
19044	2 ,,	1 ,,	,,
18773	Control	·5 c.c.	Died, B.Q.
19085	,,	·7 ,,	,,

EXPERIMENT No. 4. (S. 3415.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 4.

Sheep No.	Vaccine Subcutaneously, 28/2/28.	Virus Intramuscularly, 10/4/28.	Results.
19207...............	2 c.c.	1 c.c.	Lived.
19211...............	2 ,,	1 ,,	,,
19213...............	2 ,,	1 ,,	,,
19738...............	2 ,,	1 ,,	,,
19133...............	2 c.c. intramusc.	1 ,,	,,
19197...............	2 c.c.	1 ,,	,,
19231...............	2 ,,	1 ,,	,,
19204...............	2 ,,	1 ,,	Died, B.Q.
18733...............	Control	·5 c.c.	Died, B.Q.
19085...............	,,	·7 ,,	,,

One sheep died from black-quarter. Sheep Nos. 19133, 19197, were injected intramuscularly with no difference noted in the immunity provided.

EXPERIMENT No. 5. (S. 3467.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 5.

Sheep No.	Vaccine Subcutaneously, 16/4/28.	Virus Intramuscularly, 3/5/28.	Result.
19191...............	2 c.c.	1 c.c.	Lived.
19195...............	2 ,,	1·5 ,,	,,
19214...............	2 ,,	1·5 ,,	,,
19236...............	2 ,,	2 ,,	,,
18757...............	Control	1 c.c.	Died, B.Q.

EXPERIMENT No. 6. (S. 3475.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 6.

Sheep No.	Vaccine Subcutaneously, 17/4/28.	Virus Intramuscularly, 3/5/28.	Result.
19161...............	1 c.c.	1 c.c.	Lived.
19173...............	1 ,,	1 ,,	,,
19232...............	2 ,,	1 ,,	,,
19248...............	2 ,,	1 ,,	,,
18730...............	Control	·5 c.c.	Died, B.Q.
18757...............	,,	·7 ,,	,,

EXPERIMENT No. 7. (S. 3540.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 7.

Sheep No.	Vaccine Subcutaneously, 12/6/28.	Virus Intramuscularly, 3/7/28.	Result.
19693	1 c.c.	2 c.c.	Lived.
19691	2 ,,	3 ,,	,,
19696	2 ,,	4 ,,	,,
19712	1 ,,	2 ,,	,,
19714	2 ,,	3 ,,	,,
19717	2 ,,	4 ,,	,,
19726	1 ,,	2 ,,	,,
19728	2 ,,	3 ,,	,,
19730	2 ,,	4 ,,	,,
19703	1 ,,	2 ,,	,,
19706	2 ,,	3 ,,	,,
19709	2 ,,	4 ,,	Died, B.Q.
19954	Control	1 c.c.	Died, B.Q.
19968	,,	2 ,,	,,

EXPERIMENT No. 8. (S. 3587.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 8.

Sheep No.	Vaccine Subcutaneously, 30/7/28.	Virus Intramuscularly, 14/8/28.	Result.
20191	5 c.c.	1 c.c.	Lived.
20753	5 ,,	1·5 ,,	,,
20361	5 ,,	2 ,,	,,
20543	5 ,,	2·5 ,,	,,
20547	5 ,,	3 ,,	,,
20445	5 ,,	3·5 ,,	,,
20891	Control	·7 c.c.	Died, B.Q.
20376	,,	1 ,,	Lived.

EXPERIMENT No. 9. (S. 3607.)

To test the efficacy of Black-Quarter Formalinized Vaccine, Batch No. 9.

Sheep No.	Vaccine Subcutaneously. 30/8/28.	Virus Intramuscularly, 11/9/28.	Result.
20819	2 c.c.	1 c.c.	Lived.
20926	2 ,,	2 ,,	,,
20968	5 ,,	3 ,,	,,
20977	5 ,,	4 ,,	,,
20985	5 ,,	5 ,,	,,
20997	2 ,,	1 ,,	,,
21009	2 ,,	2 ,,	,,
21010	5 ,,	3 ,,	,,
21019	5 ,,	4 ,,	,,
21021	5 ,,	5 ,,	,,
21139	Control	17 c.c.	Died, B.Q.
21153	,,	1 ,,	,,

Summary.—Of the 62 sheep injected with formalinized vaccine, two died of black-quarter. Some of the immunized sheep withstood up to 12 minimum lethal doses. The intervals between the injection of vaccine and the test for immunity varied from 13 to 50 days.

No difference in the resultant immunity could be noted when the vaccine was injected intramuscularly instead of subcutaneously.

As the vaccine had proved itself highly efficient against the rather severe laboratory test, it was decided to give it a trial against outbreaks of black-quarter in the field. Accordingly when an outbreak of black-quarter resulting in the loss of five calves was notified in the Pretoria District, all the calves on the farm, 110 in all, were inoculated with formalinized vaccine. On the 12th September, 1927, one year later, no fresh cases of black-quarter had occurred.

COMPARATIVE TESTS BETWEEN FILTRATE AND FORMALINIZED VACCINE.

Before adopting the formalinized method for the preparation of black-quarter vaccine on a large scale (approximately quarter of a million doses are issued annually), and discarding the filtrate method, a series of experiments were carried out to determine which gave the greater degree of immunity.

EXPERIMENT No. 10. (S. 2958.)

Sheep No.	Vaccine Subcutaneously, 3/2/27.	Virus Intramuscularly, 20/2/27.	Result.
15474	2 c.c. filtrate	1 c.c.	Lived.
15575	5 ,,	1·5 ,,	,,
15460	10 ,,	3 ,,	,,
15530	2 c.c. formalinized vaccine	1 c.c.	Lived.
15584	5 ,, ,,	1·5 ,,	,,
15780	10 ,, ,,	3 ,,	,,
15846	Control	·7 c.c.	Died, B.Q.
15852	,,	1 ,,	,,

No difference could be noted between the filtrate and formalinized vaccine when tested up to 3 M.L.D.'s. It was therefore decided to increase the dose of virulent culture. See following experiment.

M'Ewen, 1926, found that formalinized cultures gave better results than filtrate from a 48-hour culture, four out of five sheep dying from the filtrate lot, whereas none died from the formalinized lot. The filtrate, however, used in these experiments is the improved filtrate as described in the eleventh and twelfth Reports of the Director of Veterinary Education and Research, and contains a more powerful aggressin than one would find in a 48-hour culture.

EXPERIMENT No. 11. (S. 3066.)

Sheep No.	Vaccine, 10/5/27.	Virus, 2/6/27.	Result.
15841	2 c.c. subc. filtrate	1 c.c. intram.	Lived.
7884	2 ,,	2 ,,	,,
8565	2 ,,	3 ,,	,,
10896	2 ,,	4 ,,	,,
10914	2 ,,	5 ,,	,,
14524	2 ,,	6 ,,	,,
16554	2 ,,	6 ,,	,,
16583	2 ,,	8 ,,	Died, B.Q.
16592	2 ,,	10 ,,	Lived.
16625	2 ,,	12 ,,	,,
15603	2 c.c. subc. formalinized vaccine	1 c.c. intram.	Lived.
15631	2 ,, ,,	2 ,,	,,
15652	2 ,, ,,	3 ,,	,,
15789	2 ,, ,,	4 ,,	,,
15803	2 ,, ,,	5 ,,	,,
15844	2 ,, ,,	6 ,,	,,
16632	2 ,, ,,	6 ,,	,,
16659	2 ,, ,,	8 ,,	,,
16706	2 ,, ,,	10 ,,	,,
16714	2 ,, ,,	12 ,,	,,
15610	Control	1 c.c. intram.	Died, B.Q.
15612	,,	1·5 ,,	,,

In this experiment one sheep of the filtrate lot died from black-quarter on receiving 8 M.L.D.'s. None of the formalinized lot died. No great difference between the two vaccines could be noted here, both protecting up to 12 M.L.D.'s. In this experiment the same filtrate and formalinized vaccine as used in the previous experiment was used.

EXPERIMENT No. 12. (S. 3467.)

Sheep No.	Vaccine, 16/4/28.	Virus, 3/5/28.	Result.
19246...............	2 c.c. subc. filtrate	1 c.c. intram.	Lived.
19278...............	2 ,, ,,	1·5 ,,	,,
19285...............	5 ,, ,,	1·5 ,,	,,
18754...............	5 ,, ,,	2 ,,	Died, B.Q.
19191...............	2 c.c. formal. vaccine	1 c.c. intram.	Lived.
19195...............	2 ,, ,,	1·5 ,,	,,
19214...............	2 ,, ,,	1·5 ,,	,,
19236...............	2 ,, ,,	2 ,,	,,
18757...............	Control	1 ,,	Died, B.Q.

Here again one of the filtrate lot died, receiving 2 M.L.D.'s, whereas none of the formalinized lot died. In this experiment the filtrate and formalinized vaccine were prepared from a fresh batch.

EXPERIMENT No. 13. (S. 3607.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
20819...............	2 c.c. formal. vaccine	1 c.c.	Lived.
20926...............	2 ,, ,,	2 ,,	,,
22968...............	5 ,, ,,	3 ,,	,,
20977...............	5 ,, ,,	4 ,,	,,
00985...............	5 ,, ,,	5 ,,	,,
21085...............	2 c.c. *filtrate.*	1 c.c.	Lived.
21089...............	2 ,,	2 ,,	Died, B.Q.
21091...............	5 ,,	3 ,,	Lived.
21092...............	5 ,,	4 ,,	Died, B.Q.
21145...............	5 ,,	5 ,,	,,
21139...............	Control	·7 c.c.	Died, B.Q.
21153...............	,,	1 ,,	,,

The formalinized vaccine and filtrate were prepared from a fresh batch after three days' growth. In this experiment the formalinized vaccine proved itself superior in immunizing value to the filtrate, none of the formalinized lot dying, whereas three out of five of the filtrate lot died of black-quarter.

Summing up, it is evident that the formalinized vaccine gives a better immunity than the filtrate and is a more stable product, for, although the filtrate gave a good protection in Experiment No. 11, it failed in Experiment No. 13. Of the 22 sheep injected with formalinized vaccine, all survived the virulent dose. Of the 22 sheep injected with filtrate, five succumbed to black-quarter.

As different authors, Kitt, Okuda (1925), Kojima (1923), Cordier (1926), Berg (1918), Leclainche and Vallée (1923), McEwen (1926), had used different media in the preparation of black-quarter vaccine with the idea of obtaining a more powerful aggressin, an experiment was carried out to determine whether there was any difference when black-quarter was grown in—

(*a*) V. Hibler's medium for 48 hours and the emulsion treated with .4 per cent. formaldehyde solution.
(*b*) Liver-liver broth and the emulsion treated as in (*a*).
(*c*) Shell diffusion medium as described by McEwen and the emulsion treated as in (*a*).

EXPERIMENT No. 14. (S. 3523.)

Sheep No.	Vaccine Subcutaneously, 30/5/28.	Virus Intramuscularly, 14/6/28.	Result.
14577...............	2 c.c. Hibler's medium	1 c.c.	Lived.
18097...............	2 ,, ,,	2 ,,	,,
18182...............	2 ,, ,,	3 ,,	,,
18189...............	2 c.c. liver-liver broth	1 c.c.	Lived.
18207...............	2 ,, ,,	2 ,,	,,
18208...............	2 ,, ,,	3 ,,	,,
18285...............	2 c.c. shell diffusion	1 c.c.	Lived.
18351...............	2 ,, ,,	2 ,,	,,
18775...............	2 ,, ,,	3 ,,	,,
19744...............	Control	1·5 c.c.	Died, B.Q.

Apparently no very marked difference as regards immunizing power is present when either v. Hibler's, liver-liver broth, or shell diffusion medium are used for the preparation of formalinized vaccine and for preparation of vaccine on a large scale. Liver-liver broth is the most practical.

To Note the Effect of Repeated Additions of Glucose to the Vaccine.

As described previously, 1 per cent. glucose is added to the medium in the preparation of the vaccine. After two to three days' growth, the glucose is exhausted by the growing bacilli. On readdition of 1 per cent. glucose to the medium and adjustment of the P^h to 8.4, vigorous growth once more occurs. Readjusting the medium in this manner three times at intervals of three days each, and drawing off a quantity of the emulsion after each period of growth, an experiment was carried out to determine whether there was any marked difference in degree of immunity between the emulsions drawn off at varying intervals. The emulsions were all treated with .4 per cent. formalin as soon as they were withdrawn from the flask.

EXPERIMENT No. 15. (S. 3540.)

Sheep No.	Vaccine Subcutaneously, 12/6/28.	Virus Intramuscularly, 3/7/28.	Result.
19693	1 c.c. 3 days' growth	3 c.c.	Lived.
19691	2 ,, ,,	4 ,,	,,
19703	1 c.c. 6 days' growth	3 c.c.	Lived.
19706	2 ,, ,,	4 ,,	,,
19712	1 c.c. 9 days' growth	3 c.c.	Lived.
19714	2 ,, ,,	4 ,,	,,
19726	1 c.c. 12 days' growth	3 c.c.	Lived.
19728	2 ,, ,,	4 ,,	,,
19954	Control	1 c.c.	Died, B.Q.
19968	,,	2 ,,	,,

From the experiment it is obvious that three days' growth is sufficient to give the formalinized vaccine sufficient immunizing power and there is no marked difference between three days' and 12 days' growth. In other words, one addition of glucose is sufficient.

No experiment was carried out to standardize the opacity of the emulsion as issued for vaccine. When the flasks containing the inoculated media had been removed from the incubator after three days' growth, they were vigorously shaken round, allowed to stand for half an hour, and the supernatent fluid containing the bacilli and spores siphoned off. Very little sediment is thus obtained in the vaccine, and the needle of the syringe will never become blocked when vaccination is carried out, providing a medium-sized needle is used.

TO DETERMINE THE LENGTH OF DURATION OF THE IMMUNIZING PROPERTIES OF FORMALINIZED VACCINE STORED AT ROOM TEMPERATURE IN ORDINARY LIGHT.

For practical purposes when vaccine is prepared on a large scale, it is necessary to determine how long the vaccine can be stored without losing its immunizing power. Accordingly a few batches were stored at room temperature in ordinary light for six and seven months respectively.

Vaccine Batch No. 3, which was prepared on the 20.2.28 (see Experiment No. 3), was retested on the 20.8.28, six months later.

EXPERIMENT No. 16. (S. 3607.)

Sheep No.	Vaccine Subcutaneously, 20/8/28.	Virus Intramuscularly, 11/9/28.	Result.
20997...............	2 c.c. 6 Months old.	1 c.c.	Lived.
21009...............	2 ,,	2 ,,	,,
21010...............	5 ,,	3 ,,	,,
21019...............	5 ,,	4 ,,	,,
21021...............	5 ,,	5 ,,	,,
21139...............	Control	·7 c.c.	Died, B.Q.
21153...............	,,	1 ,,	,,

Result.—Batch No. 3 retested six months later had still retained its full immunizing power.

EXPERIMENT No. 17. (S. 3469.)

Vaccine Batch No. 2 (see Experiment No. 2) retested seven months later.

Sheep No.	Vaccine, 16/4/28.	Virus, 3/5/28.	Result.
19163.............	2 c.c. subcut. 7 months old	1 c.c. intramuscularly	Died, B.Q.
19171.............	2 ,, ,,	1·5 ,, ,,	Lived.
19179.............	5 ,, ,,	1·5 ,, ,,	,,
19196.............	5 ,, ,,	1 ,, ,,	,,
18757.............	Control	1 c.c. intramuscularly	Died, B.Q.

Result.—One out of four immunized sheep died from black-quarter. The vaccine apparently had become slightly weaker at seven months' storage.

EXPERIMENT No. 18. (S. 3629.)

Since the formalinized vaccine prepared from cultures of the black-quarter bacilli had yielded such good results, it was interesting to determine the effect of formalin on the natural aggressin and bacilli derived from the animal itself.

A sheep was injected intramuscularly with .7 c.c. virulent black-quarter culture in the left thigh. As soon as it succumbed, the area around the seat of inoculation was cut open and the blood and muscle juice collected to which .4 per cent. formalin was added. 48 Hours later the fluid was injected into sheep to test for immunity.

Sheep No.	Vaccine Subcutaneously, 17/9/28.	Virus Intramuscularly, 3/10/28.	Result.
15915	1 c.c.	1 c.c.	Lived.
15996	1 ,,	2 ,,	,,
16013	1 ,,	3 ,,	,,
15922	1 ,,	4 ,,	,,
19534	2 c.c.	5 c.c.	Died, B. Q.
19548	2 ,,	6 ,,	Lived.
19550	2 ,,	7 ,,	,,
19565	2 ,,	8 ,,	,,
19582	5 c.c.	10 c.c.	Lived.
15983	5 ,,	12 ,,	,,
21084	Control	1 c.c.	Died, B.Q.
21017	,,	1·5 ,,	,,

Result.—All four sheep injected with 1 c.c. vaccine survived. Of the four sheep injected with 2 c.c. vaccine, one died at 5 M.L.D.'s, the other three protecting up to 6, 7, and 8 M.L.D.'s. Of the two sheep injected with 5 c.c. vaccine, both immunized up to 10 and 12 M.L.D.'s.

This is therefore a rapid and easy method of preparing black-quarter natural aggressin within 48 hours, which gives a very strong immunity. No filtration or centrifugalization is necessary, and the vaccine contains the sterile bacilli in addition to the natural aggressin present in the muscle juices and blood.

TO DETERMINE THE NATURE OF THE IMMUNITY PRODUCED IN SHEEP BY BLACK-QUARTER FORMALINIZED VACCINE.

The fact that the black-quarter bacilli while growing liberate a substance into the surrounding medium has long been made use of in the immunization against black-quarter. This product, the so-called aggressin or germ-free filtrate, has, by its superior immunizing properties, replaced the older method of using bacillary emulsions attenuated in different ways.

Immunity in black-quarter can be produced by the use of the bacilli alone or by the use of the products of the bacilli, i.e. the filtrate alone. In the use of formalinized vaccine both these factors are brought into use.

The following series of experiments were carried out to determine the nature of the action of the formalin on the bacilli and on the filtrate in a formalinized emulsion of black-quarter.

EXPERIMENT No. 19. (S. 3499.)

To show the Immunity produced by the Germ-free Filtrate alone.

Sheep No.	Vaccine, 8/5/28.	Virus, 29/5/28.	Results.
19167.........	5 c.c. filtrate subcutaneously	1 c.c. intramuscularly	Lived.
19208.........	5 ,, ,,	1·5 ,, ,,	,,
19209.........	5 ,, ,,	2 ,, ,,	,,
19218.........	5 ,, ,,	2·5 ,, ,,	,,
19530.........	Control	1 c.c. intramuscularly	Died, B.Q.

EXPERIMENT No. 20. (S. 3582.)

To show the Immunity produced by the Bacili alone.

A 48-hour culture of black-quarter was centrifuged for 15 minutes at high speed and washed with normal saline. This operation was repeated six times until it could reasonably be assumed that the bacilli had been washed free of all its culture fluid.

Sheep No.	Vaccine, 25/7/28.	Virus, 14/8/28.	Result.
20874.........	5 c.c. washed bacilli emuls.	1 c.c. intramuscularly	Lived.
20794.........	5 ,, ,,	1 ,, ,,	,,
20789.........	10 ,, ,,	2 ,, ,,	,,
20883.........	10 ,, ,,	2 ,, ,,	,,
20843.........	Control	1 c.c. intramuscularly	Died, B.Q.

The washed emulsion naturally contains a large number of spores in addition to bacilli, but for brevity "bacilli" instead of "bacilli and spores" is used throughout the paper.

TO SHOW THE ACTION OF FORMALDEHYDE ON BLACK-QUARTER FILTRATE WHICH WAS FILTERED THROUGH THE SEITZ FILTER AND TESTED TO SHOW THAT IT WAS GERM-FREE.

Two sheep were injected with filtrate to which .4 per cent. formaldehyde was added and left in contact for 14 days. Two sheep were injected with ordinary filtrate. The filtrate was prepared from a three days' growth in liver-liver broth medium.

EXPERIMENT No. 21. (3468, 3467.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
19147.........	5 c.c. filtrate & 4 % formald.	1·5 c.c.	Lived.
19159.........	5 ,, ,,	2 ,,	,,
19285.........	5 c.c. filtrate	1·5 ,,	Lived.
18754.........	5 ,,	2 ,,	Died, B.Q.
18757.........	Control	1 c.c.	Died, B.Q.

From the experiment it appeared as if the formaldehyde had exerted a beneficial action on the filtrate.

EXPERIMENT No. 22. (3566.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
20408.........	2 c.c. filtrate & ·4 % formal.	1 c.c.	Lived.
20360.........	2 ,, ,,	2 ,,	,,
20396.........	2 c.c. filtrate	1 c.c.	Died, B.Q.
20420.........	2 ,,	2 ,,	,,
20503.........	Control	1 c.c.	Died, B.Q.

A fresh batch of vaccine was prepared for the above experiment and a similar dose of filtrate injected, to accentuate if possible the difference. From the experiment it appears as if the formaldehyde has a marked beneficial effect on the filtrate. The experiment was repeated with different batches of filtrate with not such marked results as in the two previous experiments. No definite conclusion could be formed as to whether the action of formaldehyde on a weak aggressin would change it into a stronger aggressin.

In the formalinized vaccine the formaldehyde naturally exerts its action both on the bacilli and the products of the bacilli present in the emulsion, namely, the aggressin. To show that there is quite an appreciable amount of aggressin present, the formalinized vaccine was filtered through the Seitz filter and the germ-free filterate injected into sheep.

EXPERIMENT No. 23. (S. 3475.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
19161.........	2 c.c. filtrate	1 c.c.	Lived.
19173.........	2 ,,	1·5 ,,	,,
19232.........	5 ,,	1·5 ,,	,,
19248.........	5 ,,	2 ,,	,,
18757.........	Control	1 c.c.	Died, B.Q.

As it was uncertain whether the prolonged action of the formaldehyde on the aggressin would cause a certain loss in immunizing value, .4 per cent. formaldehyde was added to the filtrate and left in contact for five months before testing for immunity. The formalinized filtrate was compared with filtrate five months old to which no formaldehyde had been added.

EXPERIMENT No. 24. (S. 3498.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
19249.........	5 c.c. *filtrate and* ·4 % *form.*	1 c.c.	Lived.
19308.........	5 ,, ,,	1·5 ,,	,,
18737.........	5 ,, ,,	1·5 ,,	,,
18755.........	5 ,, ,,	2 ,,	,,
18924.........	5 c.c. *filtrate*	1 c.c.	Lived.
19121.........	5 ,,	1·5 ,,	,,
19216.........	5 ,,	1·5 ,,	,,
19242.........	5 ,,	2 ,,	,,
19530.........	Control	1 c.c.	Died, B.Q.

Results.—The formaldehyde left in contact with the filtrate for five months had no deteriorating effect on the filtrate, and the immunity produced was as good as that of the ordinary filtrate.

The following point to determine was the action of the formaldehyde on the bacilli present in the formalinized emulsion. To do this a 48-hour culture was prepared, the bacilli washed in normal saline after being centrifuged for 15 minutes. The operation was repeated six times until it could be reasonably assumed that the bacilli had been washed clear of all culture fluid. To ensure that the washed bacilli were devoid of any aggressin, 20 c.c. was inoculated into sheep without any harmful effect. .4 per cent. formaldehyde was added to the washed bacillary suspension in saline and left for 14 days before inoculating the sheep to test for immunity. Four other sheep received washed bacilli in saline suspension to which no formaldehyde had been added.

EXPERIMENT No. 25. (S. 3543.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
19690.........	5 c.c. *washed emul.* & ·4 % *F.*	3 c.c.	Lived.
19780.........	5 ,, ,,	4 ,,	,,
19786.........	5 ,, ,,	3 ,,	,,
19809.........	20 ,, ,,	4 ,,	,,
19747.........	5 c.c. *washed emulsion*	3 c.c.	Lived.
19753.........	5 ,, ,,	4 ,,	,,
19754.........	5 ,, ,,	3 ,,	,,
19751.........	20 ,, ,,	4 ,,	,,
19954.........	Control	1 c.c.	Died, B.Q.

Result.— Washed bacilli sterilized with .4 per cent. formaldehyde immunized as well as washed bacilli to which no formaldehyde had been added.

To show that no immunizing substance had been liberated from the washed bacillary suspension by the action of the formaldehyde, a washed bacillary suspension in saline was prepared as before, .4 per cent. formaldehyde added and left in contact for three weeks. The suspension was then filtered through a small Seitz filter to retain all bacillary bodies and the germ-free filtrate injected into sheep.

EXPERIMENT No. 26. (S. 3607.)

Sheep No.	Vaccine Subcutaneously, 20/8/28.	Virus Intramuscularly, 11/9/28.	Result.
21025.........	5 c.c. filtrate	1 c.c.	Died, B.Q.
21032.........	5 ,, ,,	2 ,,	,,
21046.........	10 ,, ,,	2 ,,	,,
21078.........	10 ,, ,,	3 ,,	,,
21153.........	Control	1 c.c.	Died, B.Q.

Result.—No immunizing substance is present in the surrounding fluid. The immunizing property when formalinized washed bacilli produce immunity fust therefore be present within the bacillary body itself, and is only liberated within the animal body probably when the bacilli are broken up and ingested by phacocytes.

Summary.—From the experiments it is clear that two distinct factors operate in producing immunity in black-quarter formalinized vaccine, viz., the aggressin present in the fluid and, secondly, the sterile bacilli. When formalinized washed bacilli produce immunity, the immunizing substances are present within the bacilli and only liberated when the bacilli are absorbed and broken up within the animal body. The black-quarter bacilli therefore contain what may be called an " endo-aggressin " as well as being able to produce an " exo-aggressin " and when the formalinized emulsion is injected in reality a double immunity is produced.

II.—Immunization against " Vibrion septique."

Before formaldehyde had been used to detoxicate cultures of *Vibrion septique*, the filtrate had been in use for immunization against *Vibrion septique*. The filtrate was prepared in the same way as black-quarter filtrate, but usually marked toxic swellings appeared in the inoculated region. On heating the filtrate to 65° C. for half an hour to destroy the toxin which causes the swellings, the immunizing properties in the filtrate are also destroyed as shown in the following experiment:—

EXPERIMENT No. 27. (S. 2862.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
15132.........	5 c.c. filtrate	·2 c.c.	Lived.
15135.........	10 ,,	·4 ,,	,,
15170.........	5 c.c. filtrate at 65° for ½ hr.	·2 c.c.	Died, V.S.
15172.........	10 ,, ,,	·4 ,,	,,
15772.........	Control	·2 c.c.	Died, V.S.

Result.—The two sheep which had received the ordinary filtrate survived, whereas the two sheep which had received the filtrate heated to 65° C. for half an hour both died. The filtrate therefore is unsatisfactory for inoculation against *Vibrion septique.* The experiment was repeated several times using different strains of vibrion septique with the same result. The preparation of *Vibrion septique* formalinized vaccine is the same as that for black-quarter, and the vaccine can safely be used after allowing the formaldehyde to act for one week at 37° to 40° C. in the incubator.

A few batches were prepared and tested for efficacy.

EXPERIMENT No. 28. (S. 3361.)

To Test the Efficacy of Vibrion Septique Formalinized Vaccine, Batch No. 1.

Sheep No.	Vaccine Subcutaneously, 6/1/28.	Virus Intramuscularly, 15/2/28.	Result.
17549	1 c.c.	·5 c.c.	Lived.
17593	1 ,,	·5 ,,	,,
17613	1 ,,	·5 ,,	,,
19198	Control	·3 c.c.	Died, V.S.

EXPERIMENT No. 29. (S. 3541.)

Batch No. 2.

Sheep No.	Vaccine Subcutaneously, 13/6/28.	Virus Intramuscularly, 28/6/28.	Result.
19739	5 c.c.	1 c.c.	Lived.
19741	5 ,,	1 ,,	,,
19944	Control	·5 c.c.	Died.

As a number of outbreaks of malignant oedema had occurred during the past year, especially during the sheep-shearing season, it was decided to incorporate both vibrion septique and black-quarter in one vaccine. On account of the size of the country much time is usually wasted before muscle specimens sent to the Laboratory can be properly tested and diagnosed for black-quarter or malignant oedema. As both black-quarter and malignant oedema occur in sheep, inoculation with the double vaccine can be carried out immediately an outbreak resembling black-quarter or malignant oedema occurs. Where black-quarter or malignant oedema has occurred in the past, it is advisable to inoculate the sheep a week or two before shearing commences. Black-quarter and vibrion septique are grown in separate flasks as described previously and .4 per cent. formaldehyde added. After one week in the incubator at 37° C., on bottling the vaccine in 100 c.c. bottles 50 c.c. of the black-quarter vaccine are run into the bottle and then 50 c.c. of the malignant oedema vaccine. The dose for sheep is 2 c.c. of the mixed vaccine, after thoroughly shaking the bottle.

A few batches of the mixed black-quarter and malignant oedema vaccine were prepared and tested.

EXPERIMENT No. 30. (S. 3276.)

Sheep No.	Vaccine Subcutaneously, 7/11/27.	Virus Intramuscularly, 22/11/27.	Result.
17415.........	2 c.c. mixed B.Q. and V.S.	2 c.c. B.Q.	Lived.
17481.........	2 ,, ,,	2 ,,	,,
17716.........	Control, B.Q.	2 c.c. B.Q.	Died, B.Q.
		25/11/27.	
—	—	1 c.c. V.S.	Lived.
—	—	1 ,,	,,
17546.........	Control, V.S.	1 c.c. V.S.	Died, V.S.

Result.—Both the sheep were immunized against B.Q. and V.S.

EXPERIMENT No. 31. (S. 3379.)

Batch No. 2.

Sheep No.	Vaccine Subcutaneously, 19/1/28.	Virus Intramuscularly, 6/2/28, B.Q.	Result.
17657.........	2 c.c. mixed B.Q. and V.S.	3 c.c. B.Q.	Lived.
17659.........	2 ,, ,,	3 ,,	,,
17682.........	2 ,, ,,	3 ,,	,,
17669.........	2 ,, ,,	3 ,,	Died. B.Q.
18913.........	Control, B.Q.	3 c.c. B.Q.	Died, B.Q.
		15/2/28, V.S.	
—	—	·5 c.c. V.S.	Lived.
—	—	·5 ,,	,,
—	—	·5 ,,	,,
—	Control, V.S.	·3 c.c. V.S.	Died, V.S.

Result.—The vaccine was first tested against black-quarter and nine days later against vibrion septique. One sheep died from black-quarter and the other three were immunized against both black-quarter and vibrion septique.

As shown before in the case of black-quarter, the immunity produced by the use of formalinized vibrion septique vaccine is also a double immunity. The *Vibrion septique* bacilli washed free of its toxin and suspended in saline produce immunity. Similarly the washed bacilli sterilized by the addition of .4 per cent formaldehyde produce immunity.

EXPERIMENT No. 32. (S. 3358.)

Sheep No.	Vaccine Subcutaneously, 5/1/28.	Virus Intramuscularly, 15/2/28.	Result.
17650.....	2 c.c. washed V.S. suspension	·5 c.c.	Lived.
17674.....	5 ,, ,,	1 ,,	,,
17677.....	2 c.c. washed V.S. suspension & ·4 % F.	·5 c.c.	Lived.
17709.....	5 ,, ,, ,,	1 ,,	,,
19198.....	Control	·3 c.c.	Died, V.S.

EXPERIMENT No. 33.

Experiment No. 32 was repeated with a freshly prepared vibrion septique saline suspension.

Sheep No.	Vaccine Subcutaneously, 19/6/28.	Virus Intramuscularly, 4/7/28.	Result.
19851.....	5 c.c. washed V.S. suspension	1 c.c.	Died, V.S.
19886.....	5 ,, ,,	1·5 ,,	Lived.
19892.....	5 ,, ,,	1·5 ,,	,,
19893.....	20 ,, ,,	2 ,,	,,
19902.....	5 c.c. washed V.S. suspension & ·4 % F.	1 c.c.	Lived.
19903.....	5 ,, ,,	1·5 ,,	,,
19911.....	5 ,, ,,	1·5 ,,	Died, V.S.
19918.....	20 ,, ,,	2 ,,	Lived.
19969.....	Control	1 c.c.	Died, V.S.

A fresh-washed vibrion septique saline suspension was prepared, four sheep receiving washed suspension and four receiving washed suspension plus .4 per cent. formaldehyde; the formaldehyde remaining in contact for seven days before the sheep were inoculated. In this experiment one sheep from the ordinary washed suspension lot died and one sheep from the washed suspension plus formaldehyde lot.

III.—Immunization against " B. welchii " and " B. oedematiens."

Of the anaerobes of the gas-gangrene type, black-quarter and vibrion septique are those most commonly met with in South Africa.

B. welchii has rarely been isolated from muscle specimens where the symptoms described can be attributed to *B. welchii* infection. *B. oedematiens* has never been isolated.

It was of sufficient interest, however, to note the action of formaldehyde on these members of the gas-gangrene group, and to prepare if possible a vaccine on similar lines to black-quarter and malignant oedema. We are indebted to the Lister Institute for providing the strains of *B. oedematiens* and *B. welchii*.

" B. OEDEMATIENS " FORMALINIZED VACCINE.

The medium used was liver-liver broth and the preparation the same as that for black-quarter formalinized vaccine, adding .4 per cent. formaldehyde. The formaldehyde was left in contact for 14 days before the vaccine was inoculated. As one inoculation of 5 c.c. of the vaccine was not always sufficient to produce a good immunity, a double inoculation with 12 days' interval was carried out and the result compared with a single inoculation.

EXPERIMENT No. 34. (S. 3412.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly, 24/4/28.	Result.
19287.............	5 c.c. (25/2/28)	1·5 c.c.	Died, B. Oed.
19202.............	5 „ (25/2/28) and (6/3/28)	2 „	Lived.
18768.............	Control	·7 c.c.	Died, B. Oed.

Sheep 19287, which had received only one inoculation, died; while sheep 19202, which had received a second inoculation 12 days later, survived.

The experiment was repeated, both sheep receiving a double inoculation of 5 c.c. vaccine with 12 days' interval.

EXPERIMENT No. 35. (S. 3412.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly, 2/6/28.	Result.
19006.......	5 c.c. (7/5/28) and 5 c.c. (19/5/28)	1·5 c.c.	Lived.
19074.......	5 „ „ 5 „	2 „	„
19600.......	Control	1 c.c.	Died, B. Oed.

A double inoculation with 12 days' interval produces a good immunity.

A single inoculation can, however, produce immunity in sheep, as the following experiment shows:—

EXPERIMENT No. 36. (S. 3521.)

In this experiment the medium used was v. Hibler's; .4 per cent. formaldehyde was added and left in contact with the emulsion for 14 days.

Sheep No.	Vaccine Subcutaneously, 30/5/28.	Virus Intramuscularly, 26/6/28.	Result.
19206...............	5 c.c.	2·5 c.c.	Lived.
19234...............	5 ,,	2·5 ,,	,,
19928...............	Control	·7 c.c.	Died, B. Oed.

"B. WELCHII" FORMALINIZED VACCINE.

With the use of *B. welchii* formalinized vaccine it was difficult to immunize sheep, even two injections of 5 c.c. failing in some cases to immunize. One injection of vaccine never proved successful.

EXPERIMENT No. 37. (S. 3522.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly, 26/6/28.	Result.
19307.......	5 c.c. (30/5/28)	2 c.c.	Died.
19677.......	5 ,,	2 ,,	,,
19319.......	5 c.c. (30/5/28) and 5 c.c. (14/6/28)	3 c.c.	Lived.
19681.......	5 ,, ,, 5 ,,	3 ,,	,,
19935.......	Control	3 c.c.	Died.

Both sheep receiving the double inoculation survived. Two inoculations are, however, not always successful, as the following experiment will show:—

EXPERIMENT No. 38. (S. 3409.)

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly, 26/4/28.	Result.
19157.......	5 c.c. (24/2/28) and 5 c.c. (23/3/28)	3 c.c.	Died.
19160.......	5 ,, ,, 5 ,,	4 ,,	Lived.
19568.......	Control	3 c.c.	Died.

The virulent culture was injected 34 days after the last injection of vaccine, and one sheep died although having received two inoculations of 5 c.c. each. In the case of *B. welchii*, formalinized cultures do not produce a very good immunity, whereas in black-quarter, vibrion septique, and *B. oedematiens* a single inoculation of a small dose is usually sufficient to establish a good immunity.

Seeking an explanation for this fact, washed cultures of *B. welchii* were prepared and two sheep injected.

EXPERIMENT No. 39.

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
19113.............	2 c.c. washed emulsion	3 c.c.	Died, B.W.
19117.............	5 ,, ,,	4 ,,	,,
19568.............	Control	3 c.c.	Died. B.W.

Similarly washed cultures of *B. welchii* were prepared and .4 per cent formaldehyde added, which was left in contact for 14 days before injecting.

EXPERIMENT No. 40.

Sheep No.	Vaccine Subcutaneously.	Virus Intramuscularly.	Result.
19255.....	5 c.c. washed emulsion and ·4 % F.	3 c.c.	Died, B.W.
19264.....	5 ,, ,,	4 ,,	,,
19568.....	Control	3 c.c.	Died. B.W.

There are, therefore, apparently very little or no immunizing properties present within the bacillary bodies themselves in contrast to black-quarter and vibrion septique, washed emulsions of which produced a good immunity. This possibly explains the need of repeated injections of formalinized cultures of *B. welchii*, the exoproducts of the bacilli apparently providing the immunity. Not enough sheep were used in this experiment to draw definite conclusions, but the results are in accordance with those of Weinberg and Prévot, who found that it was difficult to immunize small laboratory animals even with repeated injections of *B. welchii*, either washed lining or cultures killed by heat.

PART II.

Experiments in connection with the pathogenesis of Black-Quarter.

As the route of infection in black-quarter under natural conditions has never yet been clearly understood and has always been of a puzzling nature, a few experiments were carried out to determine if possible the mode of entry of the bacilli into the animal body.

When an examination of the carcase of an animal that has died of black-quarter in the field is carried out, usually no visible external skin lesions are found, excepting in cases of black-quarter in sheep, where the mode of entry is obviously through shearing or castration wounds. To determine this experimentally, sheep 15482 was snipped through the skin on the inner surface of the left thigh with an ordinary sheep-shearing implement. In the resulting wound, which was approximately half an inch long and extended through the subcutis, a drop of virulent black-quarter culture was placed. The sheep died 48 hours later from black-quarter. The wound must extend at least through the subcutis and submucosa before infection occurs, as intradermal inoculation of virulent culture does not cause any infection. Sheep 15275 received .5 c.c. virulent culture intradermally. Sheep 15640 received .5 c.c. plus .5 c.c. one inch away from the first injection. K. Wagener (1925) also could not establish infection by intradermal inoculation.

In calves, if no visible lesion is present on the skin, unless of course the wounds are so small that they are invisible to the naked eye, the other mode of entry may possibly be through the gastro-intestinal tract.

Sheep Nos. 1195 and 11244 were drenched by means of a stomach tube with 500 c.c. virulent culture, thereafter with 250 c.c., and a few days later with 500 c.c., without any harmful effect. It is obvious therefore that the black-quarter bacilli apparently do not pass through the intact mucous membrane and that some other predisposing cause must be present if the route of infection occurs via the intestinal tract. Two sheep, Nos. 19918 and 20399, were drenched with virulent black-quarter culture as follows: 200 c.c., 400 c.c., 600 c.c., 600 c.c., at intervals of five days. Two sheep, Nos. 20700 and 20742, received only one dose of 200 c.c., and sheep No. 20780 received 10 c.c., 10 c.c., 10 c.c., 10 c.c., 10 c.c. at intervals of five days. Fourteen days after the last dose per os, the sheep were tested for immunity by the injection of virulent culture intramuscularly.

EXPERIMENT No. 41. (S. 3578 and S. 3577.)

Sheep No.	Virulent Culture per Os.	Virus Intramuscularly.	Result.
19918.....	200 c.c., 400 c.c., 600 c.c., 600 c.c., 600 c.c.	2 c.c.	Lived.
20399.....	,, ,,	3 ,,	,,
20780.....	10 c.c., 10 c.c., 10 c.c., 10 c.c., 10 c.c.	2 ,,	,,
20700.....	200 c.c.	1·5 ,,	Died, B.Q.
20742.....	200 c.c.	1·5 ,,	,,
21173.....	Control	1 c.c.	Died, B.Q.
21181.....	,,	1·5 ,,	,,

As the bacilli apparently do not pass through the intact mucous membrane, the culture fluids containing the aggressin must have been absorbed through the intestinal wall and immunity produced in this manner. The two sheep which had received only one dose each per os both died, apparently not enough aggressin being absorbed at one dose to produce immunity.

A parallel experiment in which the sheep received only the germ-free filtrate was carried out at the same time.

EXPERIMENT No. 42.

Sheep No.	Germ-free Filtrate per Os.	Virus.	Result.
19886.....	200 c.c., 400 c.c., 600 c.c., 600 c.c., 600 c.c.	2 c.c.	Lived.
19892.....	,, ,,	3 ,,	,,
19903.....	10 c.c., 10 c.c., 10 c.c., 10 c.c., 10 c.c.	2 ,,	,,
19893.....	200 c.c.	1·5 ,,	,,
19902.....	200 ,,	1·5 ,,	,,
21173.....	Control	1 c.c.	Died, B.Q.
21181.....	,,	1·5 ,,	,,

In this experiment all the sheep were immunized by the absorption of aggressin through the intestinal wall, one large dose and small repeated doses being sufficient to establish a good immunity.

The only evidence of a pathogenic anaerobe passing through the intestinal wall is that of Turner (1927), who isolated a strain of *B. oedematiens* from the livers of sheep in Australia heavily infected with liver fluke. They attributed the cause to the penetration of the intestinal wall by the cercariae, the bacilli reaching the liver in this way and multiplying in the liver medium. Carrying out an experiment on similar lines, Sheep Nos. 19227, 19233, were dosed with approximately 20,000 and 30,000 larvae of *Haemonchus contortus* (wireworm) and 18 days later drenched with virulent black-quarter culture. No results were obtained.

Two sheep received small repeated doses of sodium fluoride per os. Marked irritation of the mucous membrane must have been present, as on the third day diarrhoea was noticed. The sheep were then drenched with 500 c.c . virulent black-quarter culture without any infection occurring.

Two calves, Nos. 2781, 2826, received daily 500 c.c. of virulent culture in their drinking water and 500 c.c. mixed with their food for two months. From the two preceding experiments described in sheep (experiments Nos. 41 and 42), it is evident that the amount of aggressin present in the virulent culture was absorbed through the mucous membrane of the intestinal tract and that the calves were soon immunized in this way.

As it is usually the heavier and thicker muscle groups in which the lesions of black-quarter are found, namely, along the shoulder and over the buttocks, these must be regarded as the predilection sites, and if the bacilli or spores gain entrance either through the mucous membrane or skin they usually find their way to these sites. To try and produce this condition experimentally, Calf No. 2791 was injected intramuscularly with 15 c.c. turpentine in the gluteal muscles. Three days later, when a large swelling was present over the injected region, 20 c.c. of virulent culture was injected intravenously and five days later again 40 c.c. of virulent culture intravenously without any infection being produced.

G. Sobernheim and Murakami (1928) tried to infect guinea-pigs by drenching them repeatedly with virulent culture and simultaneously bruising the muscles of the legs. Out of 33 guinea-pigs treated in this way, one died from black-quarter. It is obvious, however, that no infection can result in this way if the mucous membrane of the intestinal wall remains intact.

If washed bacilli and spores be injected alone into the thigh of a guinea-pig or sheep, nothing happens. If, however, a small amount of aggressin be injected simultaneously with the washed bacilli and spores, black-quarter infection occurs. As former experiments had shown that aggressin was absorbed through the intestinal tract, an experiment was carried out to determine whether black-quarter could be produced by giving aggressin per os and injecting washed culture subcutaneously. Sheep No. 13951 received 100 c.c. filtrate per os and 20 c.c. washed culture subcutaneously. Sheep No. 13983 received 1,000 c.c. filtrate per os and 100 c.c. washed culture subcutaneously. No infection could be produced in this way, which may be explained by the fact that the rate of absorption of the aggressin was too slow and that the concentration of aggressin by the time it reached the bacilli too low to cause the washed bacilli to become aggressive.

Experimentally therefore black-quarter could not be produced except by direct inoculation through the skin with virulent culture.

SUMMARY AND CONCLUSIONS.

With the use of formaldehyde, a simple, short, and efficient method of preparing black-quarter vaccine on a large scale is described.

Extensive tests carried out in sheep showed the stability and marked immunizing value of the vaccine.

Formalinized vaccine is more efficient than filtrate.

Immunization with the use of formalinized vaccine is due to two distinct factors: (1) the aggressin present in the fluid, i.e. the exo-products, (2) the sterile bacilli, i.e. the endo-products.

Different media used in the preparation of the vaccine have little or no effect on the resultant immunity.

There was no decrease in value of the vaccine after storage for six months in the light at ordinary room temperature.

Prolonged action of .4 per cent. formaldehyde on black-quarter filtrate at room temperature for five months had no effect on the immunizing value of the filtrate.

A rapid and efficient method of preparing black-quarter natural aggressin containing sterile bacilli is described.

A short and efficient method of preparing vibrion septique vaccine on a large scale is described similar to that of black-quarter.

A single inoculation of 2 c.c. bivalent black-quarter—vibrion septique formalinized vaccine confers immunity against both diseases and is of practical importance in South Africa.

With the use of *B. welchii* formalinized vaccine two or more inoculations are necessary to produce a good immunity.

All attempts to find the natural mode of infection in black-quarter failed except by direct entry of the organisms through the skin.

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A Note on Investigations into the Distribution of the Lamsiekte Organism

(*Cl. parabotulinum* C type).

By J. R. SCHEUBER, Dr.Med.Vet., Research Officer,
Onderstepoort.

THE presence of an organism of the Parabotulinus-type as the cause of Lamsiekte has already been demonstrated in carcase material and in fly larvae collected from decomposing carcases. This organism has been described by Robinson (1927). The tests described here were undertaken at the Lamsiekte Experimental Station, " Armoedsvlakte," Vryburg, C.P., chiefly in order to obtain some further information about the distribution of the organism producing Lamsiekte.

Taking as a parallel the wide distribution of the Botulinus A and B types in the soil of many countries, it was thought that experiments should be undertaken to see what the distribution of the Lamsiekte type of Botulinus organism was in the soil of a typical Lamsiekte farm such as Armoedsvlakte. Experiments were further devised to determine the distribution in living animals, carcase material, etc.

TECHNIQUE EMPLOYED.

As a rule, no actual isolation of the organism was attempted. The deciding factor was whether a culture made from the test material was able to produce Lamsiekte in guinea-pigs or not. For cultural work chopped meat medium [of Robertson according to Holman (1919)] was employed throughout the experiments. The doses were 2 to 3 c.c. of culture, given per os.

The results so obtained were on several occasions controlled by injecting subcutaneously into guinea-pigs sterile filtrates of positive cultures, and such filtrates also produced Lamsiekte in every case where this test was made.

Four toxic cultures were sent to Onderstepoort for bacteriological investigation. Of these, the toxins of two proved to be identical with that of *Cl. parabotulinum bovis* isolated from Armoedsvlakte carcase material, as shown by toxin antitoxin tests. The other two, though typically toxic, were apparently of another variety of the botulinus organism.

Investigations have led to the idea that there are probably several types of the botulinus organism at Armoedsvlakte and this is the subject of experimental work now being carried on here.

To obtain an indication of the reliability of the technique followed two samples of fly larvae, one of meat, and two of long bones, all collected from decomposing carcases, were tested and gave positive results.

Tests were then made of material such as soil, carcase material, faeces of living animals, etc., and these will now be discussed.

1. *Tests with Soil.*

These tests, it was hoped, would give some indication as to how far the soil would become infected from carcases and vice versa, and what the possibilities were of the carcases being invaded from the soil. Further, it was thought that, if the Lamsiekte organism generally occurred in the soil of infected farms, as had to be expected, it might be possible actually to demonstrate its presence in the soil.

The first samples of soil were taken in the carcase-camp of Armoedsvlakte, a small enclosure where all dead animals were deposited. The quantity of soil tested in each case was about 5 c.c.

The first test, made with soil collected directly underneath a decomposing carcase, was positive. A sample taken one foot away from such a carcase also produced Lamsiekte, but a second sample taken in the same spot 12 days later failed to do so. A further sample collected one foot away from the nearest carcase was negative, and a sample of soil taken at a distance of 10 yards from the nearest carcase also failed to produce the disease. All these samples were taken at a depth of ½ to 1 inch below the surface.

From the various grazing camps of Armoedsvlakte altogether one hundred and one samples of soil were examined. Forty-three of them were taken at a depth of ½ to 1 inch and fifty-eight at a depth of 3 to 4 inches; all these samples gave negative results. Two samples of soil collected at the dosing-crush and three samples of wind-blown dust also failed to give toxic cultures.

2. *Tests with Material Collected at Post-mortem Examinations.*

With very few exceptions material from every animal that died on Armoedsvlakte was taken at the time the post-mortem examination was held, and was tested in the same way, but the inoculum in these cases amounted to about 10 c.c.

The total number of animals so examined was 85, including one donkey, dead of arsenical poisoning, which gave a negative result and will not be taken into consideration. The remaining animals were all cattle. The specimens were chiefly taken from the digestive tract, namely, contents of the rumen in 78, of other stomachs in 18, of the colon in 77, and of other parts of the intestines in 33 cases. Usually there was no difference in the results obtained from material taken from different parts of the gastro-intestinal system of the same animal, but where such differences occurred they will be mentioned.

(*a*) In 26 out of these 84 cattle, Lamsiekte had been diagnosed as the cause of death, and in 11 cases all samples taken from the alimentary tract gave positive results. In one instance the liver was tested as well and gave the same results as the other four samples, taken from various parts of the gastro-intestinal tract, but the spleen from another such animal was negative.

Three animals gave toxic cultures only in one out of two tests, in two instances the contents of the colon being positive and those of the rumen negative, whilst in the third case the ruminal contents gave a toxic culture and those of the colon failed to do so.

In the remaining 12 animals no toxin-producing organism could be demonstrated by this method.

(*b*) In another 12 cattle the post-mortem appearance was such that Lamsiekte could not entirely be excluded.

For seven of these animals enteritis or gastro-enteritis was recorded as the cause of death because the inflammatory changes in the digestive tract were fairly marked and there were not sufficient data to warrant the diagnosis " Lamsiekte." Three of these animals gave positive cultures, both from the contents of rumen and colon. In the fourth one, only the ruminal contents gave a toxic culture, those of the colon were negative. The cultures from the other three animals failed to produce Lamsiekte.

In five cases vegetable poisoning was suspected as the cause of death, and those cattle were all negative.

(*c*) The remaining 46 cattle had died of causes distinctly different from Lamsiekte and in 43 of them no indication of this disease could be found by means of cultural tests either. In one case, however, the contents of the rumen as well as those of the colon were found to give toxic cultures. (This was a calf, dead of Black-Quarter, and the post-mortem examination was made about 24 hours after death.) From another calf (Black-Quarter, interim about six hours) the contents of the rumen gave a positive result, those of the colon were negative. In a third case (Black-Quarter, interim about 36 hours) the contents of the colon produced Lamsiekte, whilst those of the rumen failed to do so. These positive cultures were filtered and the sterile filtrate also produced Lamsiekte in guinea-pigs.

3. *Tests with Faeces from Living Animals.*

The positive results obtained in testing material from the digestive tract, collected at post-mortem examinations, supported the view that the presence of a toxogenic organism would be demonstrated in the alimentary tract of living animals as well. Faeces were collected from the rectum of 92 apparently healthy cattle and tested in quantities of about 10 c.c.

Fifty of these animals had been dosed with sterilized bone meal regularly and in 48 of them no toxic organism could be traced. Two, however, gave positive results.

The remaining 42 cattle were control-animals, not receiving any bonemeal at all and therefore liable to die from Lamsiekte sooner or later. Of this lot five gave toxic cultures and in one case the result was doubtful. (Twice in succession one out of two guinea-pigs dosed died, in one case five, in the other one seven days after dosing and nothing could be found to account for death.)

4. *Tests with Guinea-pig Carcases.*

Guinea-pig carcases were skinned and kept under various conditions. Such carcases were also liable to develop toxicity, even where every outside infection was excluded: Three out of nine carcases kept air-tight in sterile fruit-jars gave toxic cultures.

SUMMARY.

1. Soil was found to be infected with toxin-producing organisms underneath and very near decomposing carcases, but from over a hundred soil samples collected in various parts of "Armoedsvlakte" negative results were obtained.

2. More than half of the 26 animals which had died of Lamsiekte gave toxic cultures from the contents of the gastro-intestinal tract, whilst in cattle where death was due to causes other than Lamsiekte positive results were only obtained in three cases out of 47.

In some of these cases the contents of one part of the digestive system gave a positive result, whilst in those of another part the toxogenic organism was not traced. The liver tested in a positive and a negative case gave the same results as the contents of the alimentary tract, but the spleen of an animal where all samples from the digestive system were positive failed to give toxic cultures.

3. The contents of the rectum of 92 healthy animals gave toxic cultures in seven instances. The proportion of positive results was much lower in animals dosed with bonemeal (2 out of 50) than in control cattle (5 out of 42). This is merely an indication and would tend to show that bonemeal-fed animals do not ingest carcase material to the same extent as control ones.

4. Three decomposed guinea-pig carcases, where all infection from the outside had been excluded, also gave toxic cultures.

CONCLUSIONS.

As well as in decomposed bones and meat and in fly larvae, a toxin-producing anaerobe has been demonstrated:

(*a*) In soil underneath or near to decomposing carcases.
(*b*) In the digestive system of cattle, dead of Lamsiekte and from other causes.
(*c*) In the intestinal contents (rectum) of healthy cattle.
(*d*) In guinea-pigs.

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A More Refined Method of Bleeding Birds to obtain Serum for the Agglutination Test for Bacillary White Diarrhoea.

By R. A. ALEXANDER, B.Sc.A., B.V.Sc. (S.A.), Research Officer, Onderstepoort.

SINCE it has become apparent that the control of Bacillary White Diarrhoea is likely to become a matter of primary importance to the poultry industry in South Africa in the immediate future, and since detection of chronic carriers of *B. pullorum* by the agglutination test is at present the most powerful weapon in the hands of the veterinarian entrusted with the work, it was deemed necessary to develop a technique whereby adequate samples of serum could be collected in the field for testing purposes.

Several methods of collecting blood samples, as described in the literature, were tried, but for various reasons were considered unsatisfactory, namely:—

1. Puncturing or severing the wing vein, Vena brachialis, by means of a knife or lancet and collecting the blood in a bottle as described by Beaudette (1924). With this method it was found that, when a large number of birds had to be bled, the hands of the operator very soon became soiled with coagulated blood, to which dust, dirt, and feathers adhered, to form a tangled mass which was most unpleasant and seriously decreased the speed of the operation. The blood ran down the outer surface of the bottle, very effectively obliterating any distinguishing marks or numbers, and at the conclusion of the bleeding the array of sample bottles presented a most unsightly appearance. In addition, difficulty was experienced in cutting the vein in such a way as to cause a flow of blood strong enough to fill the bottle rapidly and at the same time to produce only slight secondary haemorrhage, so that, if a flock of birds with unpigmented plumage was being handled, the continued haemorrhage speedily caused so bloodstained an appearance as to give rise to considerable anxiety on the part of the owner.

2. Bleeding direct into a bottle by means of a syringe needle. The lumen of the wing vein, however, is so small that a very fine needle must be used, which considerably retards the rate of flow of the blood. Moreover, it is essential that needles be thoroughly washed before use on each bird, and this necessitates either maintaining a large supply or wasting much time in cleaning.

In the light of the above, steps were taken to develop a method which could be carried out easily, which did not unduly harm or disfigure the birds and which would result in obtaining adequate samples of blood if necessary to be despatched by rail or post to a laboratory for testing purposes. Eventually the following was evolved and has been used with considerable success.

Equipment.—

1. Sterile drawn glass pipettes (with blank label attached) of the nature commonly used for bacteriological work, i.e. glass tubing drawn out to a fine point at one end and having a constriction about 2 cm. from the other. Those which have given the best results were drawn from tubing with a 5 mm. bore so that the bulb of the pipette was 10 cm. long.
2. Sharp puncturing needle with a flat triangular point, i.e. a surgical lancet, though an ordinary large darning-needle has been used with good results.
3. A bottle of methylated spirits or alcohol.
4. A few small pledgets of absorbent cotton wool.
5. A block of paraffin wax of a hardness approximating cheese. This consistence was obtained by fusing approximately equal parts of paraffin wax and vaseline, the actual amounts varying with the natural hardness of the wax and the prevailing mean temperature.
6. A plumber's petrol blowlamp.

Personnel.—

1. The operator.
2. An assistant to hold the bird while being bled.
3. At least one assistant to catch the birds.

Preparatory Treatment.—

It is undesirable to chase birds about vigorously to catch them. Before the commencement of bleeding all birds should be shut up in crates or confined in the poultry-house. To prevent the formation of a cloudy serum it is advisable not to bleed immediately after the birds have been fed. In practice it has been found sufficient to remove the mash-hoppers at noon the day before bleeding and to give no food until after the operation next morning. Water may be allowed *ad lib.*

The Operation.—

Blood is drawn from the Vena brachialis, which is formed by the union of the Vena ulnaris and Vena radialis and passes over the medial surface of the humeral joint. It is easily to be seen situated immediately beneath the skin on the ventral surface of the extended wing. Instruct the assistant to hold the bird in the dorsal recumbent position with the left wing pressed firmly against his chest at a height convenient to the operator, the tail and legs controlled by the left hand and the head and neck tucked beneath the right arm. Extend the right wing, pluck the few feathers on its ventral surface in the region of the humeral joint and swab this region with a pledget of cotton wool soaked in alcohol to remove any dust or flakes of skin and also partially to sterilize the areas. While this is drying place between the lips and grip with the teeth one of the glass pipettes after breaking off the sealed end. Grasp the humerous between the thumb and forefingers of the left hand and pass a few of the terminal wing flight feathers round your left wrist so that they press against the outer surface of your forearm in an oblique direction from below upwards and outwards, thus maintaining the wing in an extended position. Place the thumb of the left hand on the brachial vein close to the shoulder joint and compress it by pressure directed against the forefinger placed below the humerus. The vein immediately becomes distended with blood, and either it or one of its tributaries may be punctured at a point close to the humeral joint. Blood runs from the wound and forms a pool in the depression on the surface of the fold of skin which fills the angle between the arm and forearm of the bird. Before any coagulation occurs suck up into the pipette sufficient blood to form a column about 7 cm. in length in the bulb. Then to prevent the escape of the blood temporarily seal off the end of the pipette by pushing it into the mould of paraffin wax, thus inserting a plug which will effectively block up the lumen. Stand the filled pipette sealed end downwards in any suitable container to allow the blood to coagulate. When coagulation is complete, pipettes may be sealed off at the drawn-out end and at the upper constriction, using the plumber's blow-lamp for the purpose. The phials of blood can easily be packed between layers of cotton wool or fluted cardboard for transport without fear of breakage.

Within 12 hours the clot has usually contracted to extrude the serum, which may be drained to the unfilled portion of the phial.

The birds may be numbered by marking with a copying (indelible) pencil on the unfeathered portion of skin immediately adjacent to the shoulder joint. These numbers will remain clearly legible for a period of 10-14 days even during rainy weather.

Precautions.—

1. When puncturing the vein hold the needle in a position vertical to the long axis of the vein. If the puncture is made in an oblique direction, the skin moves slightly when pressure is applied, so that on withdrawing the needle a flap of skin remains to form a valve which prevents the free flow of blood and a large haematoma is formed.

2. Control haemorrhage by applying pressure to the point of puncture with the finger for, say, half a minute. In practice it is found that this is seldom or never necessary since the flow of blood ceases immediately the wing returns to its normal position.

3. When sucking up the blood take care that a continuous column unbroken by air-bubbles is obtained.

Remarks.—

When the above method of obtaining samples of blood was first employed it was found that in approximately 8 per cent. of cases the bloodclot failed to contract and no serum was extruded. Subsequent investigation showed that this was due to one or more of the following reasons:—

1. Glass tubing of too fine a bore being used. The reason why this should affect contraction is not quite clear, but it probably stands in some relation to the ratio of the volume of the clot to the surface causing adhesion to the glass.

2. Inner surface of the glass pipette being freed of all dust and covered with a thin film of oil, thus materially interfering with the

process of coagulation. In the later work the glass tubing was not washed before drawing out the pipette and autoclaving, thus there remained behind a very fine layer of sterile dust.

3. Too rapid cooling of the blood sample. It was a constant observation that when bleeding was carried out on a cold winter's day a large percentage of the clots failed to contract. On such days, however, when the samples were transferred to an incubator at 37° immediately after being drawn, contraction was not interrupted. Under field conditions the samples were either placed in an ordinary egg incubator or as an alternative measure were placed on a shelf in the kitchen of the farm-house.

As a general rule, cocks and two-year-old or older birds are more difficult to bleed owing to the thicker skin forming a valve on puncturing which prevents the free flow of blood.

At the end of about 12 hours the blood has coagulated and the clots have contracted to extrude the serum, which is made available for the agglutination test by breaking off both ends of the phial and blowing out the liquid contents on to a watch glass.

It is not claimed that the method is aseptic but with reasonable care samples have remained in almost perfect condition with only slight haemolysis for a period of 14 days.

Conclusions.

By the method described samples of blood to provide more than sufficient serum for the agglutination test (Huddleson and Carlson, 1926; Runnels, Coom, Farley, and Thorp, 1927) (rapid method) are easily, quickly, and cleanly obtained from poultry in a manner commensurate with the dignity of a professional veterinarian. The birds are not affected more than they would be by simple handling, and after the operation the plumage of even a flock of White Leghorns shows little or no sign of being soiled with blood. Moreover, the phials of blood may be packed for dispatch by rail or post without fear of breakage. One criticism may be levelled against the method, namely, that it is somewhat costly, in that a comparatively large amount of glass-tubing is used in drawing pipettes which can only be used once. This cost, however, is not as great as would appear to be the case at first sight when consideration is given to the initial cost of bottles and the replacing of corks of other methods.

When first employing this technique the operator is apt to feel somewhat awkward and the method may appear complicated, but with even a little practice it is possible to bleed 30 birds an hour with comparative ease. Thus when used in conjunction with the rapid method of agglutination it is possible to bleed and test 250-300 birds in two days.

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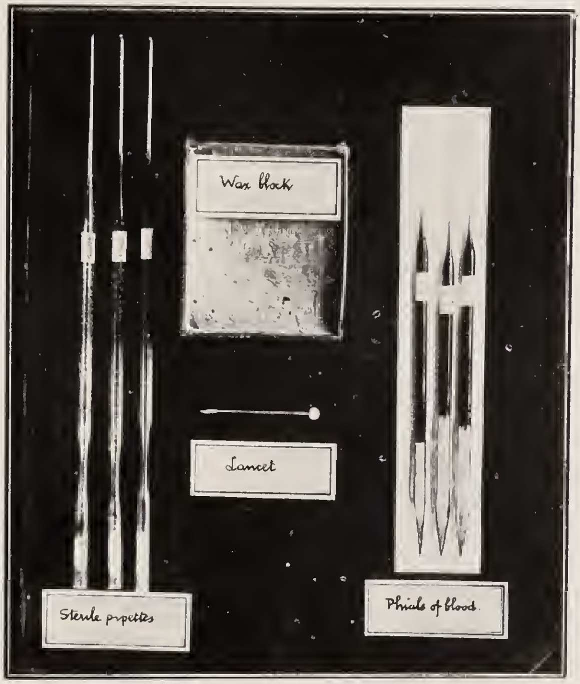

Fig. 1.
Necessary equipment.

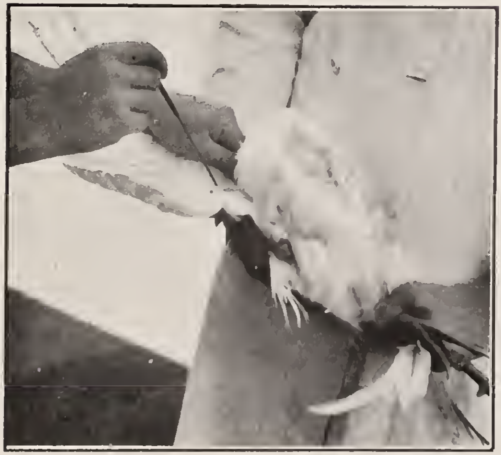

Fig. 2.
Technique of control and bleeding.

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Diseases of Domesticated Animals in South Africa due to Organisms of the *Salmonella* Group.*

Thesis accepted for the degree of Doctor of Veterinary Science by the University of Toronto.

By G. MARTINAGLIA, V.S., B.V.Sc., M.Sc., Veterinary Research Officer, Onderstepoort.

GENERAL INTRODUCTION.

WELL has it been said by Savage and White (1925), in their excellent paper on the *Salmonella* group, that "the typing of each strain is in itself a little research, demanding careful attention and, often, unstinted patience."

Since Salmon and Smith encountered *S. suipestifer*, the first member of this interesting group to be studied, the identification and interclassification of closely allied species of the genus have been problems to many a bacteriologist.

In exploring the bacteriology of this group for the first time in South Africa, the writer has availed himself of the newer knowledge of the day, contributed especially by such investigators as Jordan (1925), Savage and White (1925).

Prior to 1923, it was not known whether the organisms of the *Salmonella* group played any part in the causation of disease among domesticated animals in the Union of South Africa. Since that year the writer has been interested in diseases caused by the bacteria of the genus *Salmonella*, and to-day it can be stated safely that they are a potential factor in the production of mortality in calves, young and adult poultry, canaries in commercial aviaries, and occasionally as secondary invaders in equines during horsesickness immunization.

In presenting this article it is intended to record the presence of several diseases in domesticated animals, poultry, and canaries due to organisms of the Salmonella group, and, in addition, to record the presence of the following species:—

Disease.	*Species.*
Calf Paratyphoid.	*S. enteritidis* and *S. aertrycke.*
Tendo-vaginitis in Horses.	*S. abortivo-equinus.*
Bacillary White Diarrhoea of Chickens.	*S. pullorum.*
Fowl Typhoid.	*S. gallinarum.*
Canary Paratyphoid.	*S. aertrycke.*

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* In preparing for publication a number of minor changes have been made; illustrations and bibliography have been slightly changed and text reduced.

CALF PARATYPHOID.

Calf diseases produced by paracolon bacilli were apparently first demonstrated by Thomassen (1898), and described under the term "*Bakteraemia.*" Subsequently Poels (1899) described a disease of calves in Holland due to pseudo-colon bacilli. Later Jensen (1913) described diseases of calves as well as young cattle under the designation of Colibacillosis. Christiansen (1915) gave his experience at the Veterinary School at Copenhagen in connection with septicaemic colibacillosis of calves. He pointed out that the terms pseudocolon bacilli and paracolon bacilli, coined by Poels and Jensen respectively, are synonymous, and that the term paracolon bacilli is often used in German literature as identical with the Gärtner bacillus. In comparing his paracolon strains isolated from calves with the Gärtner laboratory strains, he could not establish any differences between them.

Meyer, Traum, and Roadhouse (1916) described an outbreak of infectious diarrhoea of hand-fed calves in California due to *B. enteritidis*.

Karsten (1921) incriminated *B. enteritidis* as the chief cause of calf paratyphoid.

Viljoen and Martinaglia, who first investigated this calf disease in the western Transvaal in 1923, have published preliminary communications (1926-1928). The writer has since then, when opportunity offered, continued his observations. Originally it was established that organisms of the *Salmonella* group played an important rôle, both as primary and secondary invaders. The main species responsible was *S. enteritidis* (*B. enteritidis*), but *S. aertrycke* was also identified in one outbreak.

Daubney (1927) reported a paratyphoid infection of calves in Kenya, due to organisms of the *S. enteritidis* type.

A report on calf typhoid has appeared in the 13th and 14th Reports of the Director of Veterinary Education and Research, in which the following aspects were discussed by Viljoen and the writer (1928):—

Locality, climatic conditions, farming conditions, enzootic diseases, animals affected, symptomaiology, post-mortem appearances, microscopic lesions, history of strain studied, bacteriology, serological differences, pathogenicity, immunity, summary and conclusions.

In this communication the writer wishes to add a few more observations, especially on the microscopic pathology, diagnosis, differential diagnosis, and to repeat passages only where such are essential.

POST-MORTEM APPEARANCES.

The most notable alteration is in the liver, this organ being enlarged, very often yellowish in colour and sometimes showing a mottled appearance; on section, the affected liver is found to be softer than normal and friable. Very often, on closer inspection, small focal necrotic areas can be seen through the capsule, and on section, these necrotic areas are found to extend throughout the parenchyma. Owing to the obvious change in the liver, many farmers have designated the conditions " liver disease " in calves.

The mucosa of the gall-bladder is often thickened, oedematous, and the bile syrupy in consistence and of a golden green colour. In most cases the spleen is swollen and the pulp softer than normal. A catarrhal enteritis is present in many cases, and in one calf, small necrotic foci were found in the mucosa of the intestines. The fourth stomach often contains a rubbery sponge-like piece of coagulated milk. Glands are often slightly enlarged. Petechiae are seen sometimes in the cortex of the kidneys.

At first the disease was believed by farmers to be a form of septic or infectious pneumonia, but well-marked pneumonic lesions are the exception rather than the rule. In some cases atelectatic areas in the lungs were met with, while in rare instances pneumonic areas, particularly of the anterior lobes, with fibrinous pleuritis, were observed.

Small abscesses were present in these areas and from them paratyphoid organisms could be isolated.

In cases where the liver is badly affected, complicated with a cholecystitis, there is usually a marked general icterus.

MICROSCOPICAL PATHOLOGY.

The most noticeable pathological changes occur in the liver, spleen, and sometimes in the lungs and lymph glands.

Liver.—In per-acute cases the nodule seems to start as a haemorrhage. There is an intense focal hyperaemia, but no leucocytosis. The liver cells in the focus undergo cloudy swelling and vacuolization. The nuclei become pyknotic, the cytoplasm reddish pink and disappears. Finally the nuclei also disappear and the focus is occupied by an influx of erythrocytes. It is only after the initial focal haemorrhage that leucocytosis takes place. There may also be a diffuse hyperaemia, where individual liver cells undergo disintegration. Some of the cells actually contain red staining globules simulating erythrocytes which appear to have entered a vacuole in the liver cell. In this way many red cells are probably destroyed, and this might account for the anaemia often encountered during paratyphoid epizootics in domesticated animals. In more protracted cases the lesions show a greater tendency towards the formation of disseminated necrotic foci. Here and there are small localized rounded or oval areas of necrosis. These are situated either close to the central vein or in any other position in the liver lobule. The surrounding liver cells are arranged in the usual columns. The margins of the necrotic foci are sharply demarcated. The surrounding tissues show a greater or lesser infiltration with neutrophiles, and round cells, but sometimes may be quite free from these.

The necrotic focus itself appears as a faintly staining amorphous mass of cell debris, containing numerous pyknotic and disintegrating nuclei. Foci vary in size, involving from three or four liver cells up to fairly large areas, covering a small part of the lobule or even the whole lobule.

Blood elements sometimes penetrate into the necrotic mass. The rest of the liver parenchyma is hyperaemic and shows more or less diffuse infiltration with neutrophiles. In many places accumulations of round cells can be seen. The multiple disseminated necrotic foci are present in most liver specimens and conform to the description already given. Some, however, present certain differences and probably represent the early stages of necrosis.

A number of neutrophiles become aggregated together, occupying areas of varying sizes. Some of the adjacent liver cells undergo disintegration, the nuclei disappear, the cytoplasm loses its structure, and changes into an amorphous mass. The neutrophiles present are incorporated into this mass and their nuclei become pyknotic, giving rise to the picture already described.

Bacteria can sometimes be distinguished at the edge of the necrotic foci and among the neighbouring liver cells. Here and there accumulations of round cells into irregular foci can be observed. They are not as a rule associated with Glisson's capsule. The necrotic foci are often found near the central vein of the lobule and either one or more may be seen round one vein. In some cases the central vein is entirely surrounded by a necrotic zone. Such a central vein may contain a thrombus continuous through the intima with a necrotic focus. Organizing thrombi can also be seen in other veins in which the association with necrotic foci is not evident. In some livers the development of the lesions from small accumulations of neutrophiles to the complete necrotic focus can be followed in its various stages.

Spleen.—This organ shows well-defined necrotic areas distributed very evenly in the pulp. The foci are fairly uniform in size and about equal in extent to an ordinary spleen follicle. These foci do not affect the follicles, but may be situated quite close to them. They consist more or less of an amorphous mass of cell debris filled with a large number of disintegrating pyknotic nuclei.

The edges are fairly sharply defined, but merge into the pulp without any intervening capsule or limiting tissue. The pulp may show the presence of fairly numerous neutrophiles.

Lung.—A few specimens examined show localized patches of congestion with neutrophilic infiltration.

Kidney.—A few small foci consisting of erythrocytes and neutrophiles are seen here and there. In some cases the tubules of the cortex contain a moderate amount of albuminoid substance.

Glands.—The glands occasionally, especially the periportal, show areas of necrosis similar to those seen in the spleen and liver.

There is a striking similarity between the human typhoid focus of the liver as illustrated in MacCallum's textbook on Pathology (1922) and paratyphoid lesions of domesticated animals. (See Figs. 3 and 4.) This similarity is also commented on by Joest (1924) in his description of what he terms "pseudo-tubercle" in *B. enteritidis* infection of calves. In a recent article on the pathology of calf paratyphoid Nieberle (1928) describes the lesions fully, and discusses the probable histogenesis of these cellular and necrotic centres.

Pathogenic Properties.—These vary greatly, depending on the age of the strains; when freshly isolated from calves suffering from the natural disease, all strains are pathogenic for small laboratory animals, while older strains that have been kept in the laboratory and sub-cultured for some time, lose their virulence very rapidly.

In small laboratory animals the following observations have been made:—

Guinea-pigs are susceptible, 1 c.c. of a 24-hour culture administered subcutaneously proving fatal in from six to ten days and intraperitoneally in from one to nine days. It was not possible to kill guinea-pigs by giving cultures per os. All the strains were also proved to be pathogenic for rabbits and white rats. On post-mortem examination of small laboratory animals killed by the organisms, the following changes are commonly observed: hyperaemia of the subcutis, omental suppuration, fibrinous exudate on the liver, peritonitis, and very often an excess of fluid in the peritoneal cavity. Necrotic areas in the liver so commonly met with in calves suffering from the natural disease, are rarely seen with the naked eye in laboratory animals.

Sheep.—Two sheep were injected with a 24-hour broth culture, one receiving 1 c.c. subcutaneously and one 1 c.c. intravenously. The one which received the subcutaneous dose developed a mild fever lasting six days and eventually recovered. The one which received the intravenous dose developed a high fever reaching 107.5° and died on the 10th day. Just before death it was suffering from diarrhoea and extreme weakness. Necropsy revealed mottled appearance of the liver, splenic tumour, and enlargement of the abdominal glands.

On microscopical examination necrotic foci similar to those seen in calf typhoid were observed, but much smaller, while the lesions in the glands were more marked and larger than those generally seen in the glands of calves.

Robinson and Lawrence (1928) mentioned a case where sheep were injected with blood from a calf with a high fever which was suspected to be suffering from heartwater. The sheep showed slight temperature reactions after injection, which suggested that these sheep must have been infected with paratyphoid organisms from the calf, as the sera of these sheep, subsequently, gave a strongly positive agglutination reaction to *S. enteritidis* of calves.

In *calves* of the susceptible age, marked disturbances that may even lead to death are observed in many cases, provided freshly isolated *S. enteritidis* strains are used in the experiments. (See temperature charts.)

DIAGNOSIS.

Diagnosis is often difficult clinically owing to the number of other calf diseases in South Africa simulating calf salmonellosis. Bacteriologically it can be diagnosed easily in the bacteriaemic stage, by running blood from the jugular vein through a sterile needle into broth and then transferring to plain agar by means of a sterile pipette.

Serologically a diagnosis can be made by taking sera from several calves in a sick herd and testing by the rapid agglutination test, using *S. enteritidis* and *S. aertrycke* as antigens. In some cases *S. enteritidis* organisms isolated from the blood of sick calves do not show an agglutination reaction with their homologous sera, in other cases an agglutination titre of 1 in 10,000 may be recorded. It would appear that in some cases the forming of agglutinins takes place slowly. Pathologically the disease can often be diagnosed by the microscopic examination of the liver.

In the field the diagnosis can be effected by finding the characteristic lesions.

DIFFERENTIAL DIAGNOSIS.

In making a diagnosis of paratyphoid of calves in the Transvaal lowveld, the presence of the following diseases should be borne in mind: White scour, anaplasmosis, large piroplasmosis (redwater), small piroplasmosis [(1) gonderiosis, (2) theileriosis], sweatingsickness, heartwater (rickettsiosis), pneumo-enteritis of unknown origin, coccidiosis, non-specific gastric obstructions (hairballs, etc.).

SUMMARY AND CONCLUSIONS.

1. *S. enteritidis* is the main organism responsible for calf paratyphoid in the Union.

Of many calf paratyphoid epizootics investigated, only once has *S. aertrycke* been incriminated as the cause.

Salmonella organisms are apparently widespread in the Union where ranching and dairying are carried on concurrently.

2. It has been shown that organisms recently isolated from calves can be highly pathogenic for this class of animal, but that fatal results are not usually produced in calves that are strong, healthy, and well nourished.

It would appear that the paratyphoid organisms are often secondary invaders, exerting their pathogenic effects in calves whose vitality has been lowered by other factors. Among these factors must be mentioned:

(*a*) Improper and irregular feeding and bad hygienic conditions which predispose to digestive disturbances.

(*b*) Infection with piroplasms and anaplasms. In the Marico District paratyphoid infection was commonly found as a complication of anaplasmosis and piroplasmosis. At Onderstepoort, paratyphoid infection was found in two cases of anaplosmosis artificially produced in splenectomized animals.

(*c*) Infection with trypanosomiasis. At Onderstepoort one such case was met with in a calf that had been artificially infected with *T. congolense.*

(*d*) Infection with *Coccidia* and *Giardia bovis* have also been diagnosed in Marico in a few cases of red scour, but paratyphoid infection has so far not been established definitely in these infections.

(*e*) Sweating sickness and heartwater.

3. Incidentally these observations led to the discovery of Koch's bodies not belonging to the developmental cycle of *Theileria parva.* These Koch's bodies may not be of any great pathogenic significance, but their occurrence in the Union constitutes a complicating factor in so far as the microscopical diagnosis of East Coast fever is concerned.

4. Apart from anti-paratyphoid vaccination, preventive measures against this calf mortality consist in practising sound methods of animal husbandry and adopting general hygienic principles. Among

the latter must be mentioned tick eradication by regular short-interval dipping continued over a long period.

5. Since the mortality occurs only during the late summer months and only in calves under four months old, it can be greatly reduced by arranging the calving period for the early winter months.

FINAL NOTE.

On visiting the Bushveld again six years later, in connection with the Marico calf disease, great improvement was noticed in calf husbandry.

Many of the farmers, acting on previous advice, had started to dip their cattle. These men have prospered, and the dreaded calf disease is no longer the terror of the Marico bushveld farmer. Heartwater is still taking a fair toll, but it was observed that dipping at seven days' interval had, in many cases, reduced to a minimum the calf mortality, which previously averaged about 70 per cent. of the yield.

A few cases of calf typhoid encountered were on farms where dipping was irregularly carried out or not at all.

The elimination of the tick-borne diseases by dipping, combined with regular feeding, vaccination, and better sanitation, seem to have reduced the incidence of calf typhoid considerably.

TENDO-VAGINITIS IN EQUINES, FOLLOWING HORSE-SICKNESS IMMUNIZATION, ASSOCIATED WITH ORGANISMS INDISTINGUISHABLE FROM SALMONELLA ABORTIVO-EQUINUS.

INTRODUCTION.

Organisms of the paratyphoid enteritidis group in horses were first encountered in Pennsylvania, by Kilborne (1893) and Theobald Smith (1893). Smith studied the organisms of two agar slants which Kilborne inoculated the previous evening, from the vagina of a mare which had aborted. He described the salient biochemical and cultural features, and referred to its membranous growth on agar as well as its wrinkled appearance near the condensation water. Subsequently outbreaks, apparently due to the same organism, were recorded by Turner* (1894), Lignières† in France and Argentine (1897), Poljakov‡ in Russia (1904), De Jong in Holland (1913), Good and Corbet in Kentucky (1913), Meyer and Boerner in Pennsylvania (1913), Dassonville and Rivière in France (1913), Lautenbach (1913) and Van Heelsbergen (1913) in Holland, Schofield in Canada (1914), Cominotti in Italy (1916), McFadyean and Edwards in England (1917), Miessner and Berge in Germany

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* In which State of the U.S.A. it is not clear.

† See Jordan (1925).

‡ See Magnusson.

(1917), Murray in Iowa (1919), and Kii, Sato, Nakamura, and Taguchi in Japan (1923). Good and Corbet suggested the name *B. abortivus-equinus* for the organisms they encountered (subsequently corrected to *B. abortivo-equinus*). Meyer and Boerner, who studied their strain at the same time as Good and Corbet, designated it *B. abortus equi*. Miessner and Berge, for logical reasons, suggested the name *Bacterium paratyphi abortus equi*. Bacteria similar to *B. abortivo-equinus* were also studied by Bassonville and Rivière, Good and Smith (1914), Schofield, and McFadyean and Edwards, all of whom considered the organisms as an etiological factor in pyaemic arthritis or joint ill in foals.

In presenting this paper, the writer wishes to record the occurrence of an organism indistinguishable from *S. abortivo-equinus* in tendo-vaginitis of horses and mules following horsesickness immunization.

OCCURRENCE.

In 1922 a number of adult horses which were undergoing horsesickness immunization at the Onderstepoort Laboratory suddenly developed tendo-vaginitis, when well on the way to recovery. The main seats of infection were the flexor tendons of the digits and bursae of the hock and fetlocks. In one instance there was an oedematous swelling on the chest, and in another the testes were the sole organs affected. The disease is generally ushered in by a sudden lameness in one or more limbs. The lameness is generally located in the hind or front fetlock region. On rare occasions it commences as a swelling of the hock joint, or upper forearms. The affected regions are, as a rule, warm and painful. At the onset of the disease the animals go slightly off their feed and lose condition rapidly. When once decubitus there is little chance of recovery. There is a degeneration of flexor tendons, especially those of the front leg; in one instance the deep flexor tendon actually rupturing. The first symptoms appear from three to six weeks after the first injection of horsesickness virus, the temperature during the infection varying from 99° to 105.57° F.

CASES STUDIED.

Of the 12 horses studied in 1922, the organism was obtained pure in nine instances, and in three associated with streptococci. Pipettes were used in the case of dead animals, and sterile trocars and canulas in live animals, in order to obtain material for bacteriological work. Fluid was obtained from the swellings and affected bursae, the contents of which varied from a serosanguinous fluid to a creamy purulent material. Good growth was generally obtained on plain and serum agar. On one occasion a young stallion developed a severe orchitis. The animal, which ultimately recovered, was castrated, and the organisms isolated in pure culture from the testes. Another animal developed a swelling of the right fore-arm and bacteriological examination showed the usual gram negative bacilli. An autogenous vaccine was immediately employed, and as a consequence the patient recovered.

The batch of sera with which these horses had been previously immunized was subjected to a very careful bacteriological examination, but the bacillus in question *was not* found.

In 1925 a similar outbreak occurred in a number of mules, also as a sequel to horsesickness immunization. One of the affected mules was selected for bacteriological work and duly chloroformed. Cultures were then made from an abscess on the lateral aspect of the front leg, about three inches below the knee. Another culture was made from the digital synovial sheath behind the fetlock. From the former site a mixed growth was obtained of a gram negative bacillus and a gram positive streptococcus. From the latter site a gram negative bacillus was obtained in pure culture. These bacilli, on further study, proved to be identical with the organisms isolated from tendo-vaginitis in horses, following horsesickness immunization.

Shortly after this, a fore-limb (mule), in good condition, received at the laboratory, proved to be a case of tendo-vaginitis, and in this case also identical organisms were isolated in pure culture from the digital synovial sheath. Again a searching examination was made for this organism in the sera from which these mules had been inoculated, but again with *negative results*. From the same batch many horses and mules had been inoculated in other localities, but in all cases with untoward results.

BACTERIOLOGY.

Morphology and Staining.—Irregular, short, plump, gramnegative rods with rounded ends varying from a coccal to an occasional long bacillary form. In freshly isolated cultures, especially when grown on serum agar, a peculiar staining is noticed. When stained with dilute carbol Fuchin it varies from deeply staining granules, with a light zone on one or both extremities, to rods, with similar lightly staining extremities. When stained with Ziehl's Carbol Fuchsin, it may even show a unipolar, bipolar, or belted appearance. After long laboratory cultivation, there is a tendency to grow in long filamentous forms; smears prepared from potato media often manifest a short rod or coccal appearance. The organism stains with all the laboratory aniline dyes.

Motility.—The organism is actively motile and possesses peritrichous flagella.

Cultural Features.—Plain Agar: On this medium the organism grows well; at first the colonies are smooth, delicate, and transparent, but from 47 hours onwards they become dull, slightly granular, and assume a characteristic concentric ringed appearance. Some colonies may show a flattened margin. The confluent growth at the condensation water becomes wrinkled. After a few days' incubation, the growth becomes dry and membranous and slightly adherent to the substratum. *Potato*: On this medium growth is moderate and not characteristic. *Gelatin*: Colonies transparent, small and circular, with slightly lobate margin, delicately concentric rings, and slightly raised centre. *Gelatin Stab*: Surface growth around needle entrance, with filiform growth along needle track. No liquefaction is produced. *Broth*: This becomes turbid from 12 to 24 hours after inoculation. With age most cultures develop a fragile papery pallicle which extends for a few mm. above the broth along the side of the tube.

Biochemical Features.—Indol is not produced. Nitrates are not reduced to nitrites. On *lead acteate agar* (Jordan) there developes no blackening along the needle track. In *litmus milk*: There is slight initial acidity followed by progressive alkalinity. *Milk* is not coagulated.

Table No. 1 shows the behaviour on carbohydrate media of strains investigated, including one American strain for comparative purposes. The last mentioned was kindly supplied by Prof. Jordan, of Chicago University.

SEROLOGICAL EXAMINATION.

The serum of horses which have passed through an attack of tendo-vaginitis has a high agglutination affinity for all local horse and mule strains, including the American one of *S. abortivo-equinus*.

The titre in these cases varies from 1 in 6,000 to 1 in 10,000. The normal agglutination titre for these organisms in horses examined in Natal and the Transvaal varies from 1-100 to 1-300, and sometimes there may be a faint agglutination in 1-400, but never higher.

A normal sheep, whose serum proved negative for all the Salmonella in our possession, was immunized with a typical strain obtained from a horse suffering with tendo-vaginitis.

It received three injections at weekly intervals. The first subcutaneous injection consisted of 5 c.c. of a 24-hour broth culture heated at 50° C. for one hour. The second injection consisted of a 3 c.c. living 24-hour broth culture. And finally a similar dose of living organisms was given intravenously. See Table II for agglutination reactions.

TABLE No. I.

Carbohydrate Reaction after 14 days' Incubation: In media consisting of 2 per cent. Peptone in Distilled Water, with 1 per cent. Andrade's Indicator.—1 per cent. of the Test Substance

	Saccharine.	Dextrine.	Irethrite.	Inulin.	Raffinose.	Salicin.	Inosite.	Nutrose.	Adonite.	Lactose.	Arabinose.	Rhamnose.	Galactose.	Glucose.	Mannite.	Sorbite.	Maltose.	Mannose.	Yylose.	Dulcite.	Laevulose.
Horse No. 15530	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
,, 15621	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
,, 15495	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
,, 15492	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
,, 14145	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
,, 15624	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Mule No. 1	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
,, 2	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
American Strain No. 196	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

TABLE

Showing Results of various Salmonella *Tested with*

Source of Origin.	Emulsions.		
		$\frac{1}{25}$	$\frac{1}{50}$
America..........	*S. abortivo–eëuinus*.............	+ + +	+ + +
Transvaal.........	*S. abortivo– eëuinus* (horse).......	+ + +	+ + +
America..........	*S. aertrycke* (Jordan)............	+ + +	+ + +
Natal.............	*S. aertrycke* (canary).............	+ + +	+ + +
Transvaal.........	*S. aertrycke* (calf)...............	+ + +	+ + +
Lister Institute....	*S. typhimurium*..................	+ + +	+ + +
America..........	*S. enteritidis* (Jordan)............	+ + +	+ + +
Transvaal.........	*S. entertidis* (calf)...............	+ + +	+ + +
Lister Institute....	*S. enteritidis* (Gòrtner)...........	+ + +	+ + +
Transvaal.........	*S. gallinarum* (Smook)...........	+ + +	+ + +
Lister Institute....	*S. gallinarum* (Harv.)............	+ + +	+ + +
Lister Institute....	*S. gallinarum* (Klein)............	+ + +	+ + +
Transvaal.........	*S. pullorum*....................	+ + +	+ + +
Lister Institute....	*S. pullorum* (W. 17).............	+ + +	+ + +
Lister Institute....	*S. pullorum* (Chick 11)...........	+ + +	+ + +
Lister Institute....	*S. paratyphi*....................	—	—
Lister Institute....	*S. schottmulleri*.................	+ + +	+ + +
America..........	*S. suipestifer*...................	—	—

SYMBOLS:—

+
+ + } = Complete agglutination.

No. II.

Local S. abortive-equinus. *Sheep-immune Serum.*

Serum Dilutions.

$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$	$\frac{1}{12800}$
+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	—	—
+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	—
+ + +	+	—	—	—	—	—	—
+ + +	+	—	—	—	—	—	—
+ + +	+ + +	++	—	—	—	—	—
+ + +	++	+	—	—	—	—	—
+ + +	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
+ + +	—	—	—	—	—	—	—
+ + +	+ + +	—	—	—	—	—	—
++	+	—	—	—	—	—	—
—	—	—	—	—	—	—	—
+ + +	+ + +	+ + +	+ + +	—	—	—	—
+ + +	+ + +	+ + +	—	—	—	—	—
+ + +	+ + +	+ + +	—	—	—	—	—
—	—	—	—	—	—	—	—
++	+	—	—	—	—	—	—
—	—	—	—	—	—	—	—

++ = Marked agglutination.
+ = Partial agglutination.
— = No agglutination.

PATHOGENICITY FOR LABORATORY ANIMALS.

These equine paratyphoid strains were not as highly pathogenic for laboratory animals as the members of the *enteritidis* and *aertrycke* varieties isolated from calves in the Transvaal.

The virulence of six freshly isolated cultures was tested on twelve guinea-pigs and six rabbits. Two guinea-pigs were injected from a 24-hour broth culture; of these one received 1 c.c. intraperitoneally; the other 1 c.c. subcutaneously from each culture. Three out of the twelve injected died. Two of these were injected intraperitoneally and one subcutaneously. The organism in question was again recovered from the heart blood, in these cases, in pure culture.

Six rabbits were injected with a 24-hour broth culture; three received 1 c.c. intravenously and three 1 c.c. intraperitoneally. One which received an intraperitoneal dose died 14 days after injection with an enlarged liver and slight peritonitis; the others showed no untoward results.

PATHOGENICITY FOR HORSES.

When administered intravenously in doses varying from 2.5 c.c. to 5 c.c. organisms are not pathogenic for horses. A temperature reaction develops, which varies from 104 to 105.5° F., but subsides again after two to four days following the injection. A slight phlebitis at the point of injection sometimes persists for several weeks.

No pregnant mares were available for inoculation to ascertain the ability of these organisms to produce abortion. When given subcutaneously, a marked swelling is generally produced, lasting, in some cases, a fortnight.

Two horses which received subcutaneously each 2½ c.c. of a 20-hour broth culture above the carpal joint, developed a severe swelling extending over the joint with resultant lameness lasting two weeks. Typical tendo-vaginitis was not produced. It is possible that the effect of the horsesickness virus on the tendons is essential before these organisms can do their maximum damage.

DIFFERENTIAL DIAGNOSIS.

Occasionally a pure streptococcic tendo-vaginitis is also met with following horsesickness immunization, but in these cases there is a tendency to abscess formation and suppuration through the skin. In tendo-vaginitis associated with the equine paratyphoid under discussion no suppuration to the exterior has been noted.

SALMONELLA PULLORUM INFECTION OF CHICKENS.

BACILLARY WHITE DIARRHOEA, which has assumed such vast economic importance in the United States of America, has perhaps received more attention than any other poultry disease. Veterinary science owes much to American investigators for many of the salient facts known about this malady, and the names of Rettger and his co-workers

(1900-19) are eminently associated with the main early discoveries in the study of bacillary white diarrhoea.

Beaudette (1925) has given an excellent review of the literature of this disease, and it is the intention of the writer merely to touch on the early history of bacillary white diarrhoea.

Rettger (1900) drew attention to septicaemia in chickens and described the causal organism later. He and Harvey (1908) definitely placed this organism in the colon typhoid group and the following year they assigned the name of *Bacterium pullorum* to the organism.

Among the early investigators of this disease, Jones (1910-12) and Gage (1911-16) also figured prominently and to-day the cycle of infection of bacillary white diarrhoea is well known to students of veterinary science. Only recently have investigators in Europe paid attention to this disease. Among these investigators Doyle (1925) is one of the foremost.

As far as is known, this disease had not been recorded in Africa until Donatien, Plantureaux, and Lestoquard (1924) contributed an interesting article on Avian typhoid in Algiers.

The organism which they studied did not ferment dulcite, an important differential sugar between *S. gallinarum* and *S. pullorum*. It would thus appear that these authors were working with a type of *S. pullorum*, for Jones has definitely shown that *S. pullorum* can be the cause of an acute infection of adult fowls as well as chickens.

In South Africa, poultry imported from America, Australia, and Europe are not tested, so the introduction of this disease was inevitable. While investigating fowl typhoid epizootics in South Africa, the existence of *S. pullorum* infection of adult poultry was always borne in mind, but was never diagnosed.

Bacillary white diarrhoea was, for the first time, bacteriologically diagnosed in South Africa in 1926. The outbreak had the following history: The owner bought a number of day-old chicks. They were sick on arrival and about 75 per cent. of them died. Later, he had bad results from the eggs of the survivors, as, after hatching, the young chicks (one to three days) invariably developed a fatal diarrhoea. The writer examined the last batch of chicks bacteriologically, those examined being from three to ten days old.

The clinical picture was that of bacillary white diarrhoea. Cultures were made from the various organs and an anaerogenous type of *S. pullorum* obtained in pure culture. Sera from four of the six hens responsible for the eggs from which these chicks were hatched were submitted to the agglutination test and were markedly positive. (See Fig. 19 on page 287.)

SYMPTOMS.

The affected chickens are drowsy and listless, standing for some time in the same position, swaying backwards and forwards. The main feature is the presence of a light-cream coloured diarrhoea of a sticky nature. A sound of pain is uttered during the act of faecal evacuation and the feathers are glued around the anal aperture.

POST-MORTEM APPEARANCES.

On post-mortem, the main feature is a slight enlargement of the liver, which displays minute grey specks, as often seen in fowl typhoid. These occur throughout the liver parenchyma. In one specimen, caseous nodules were found in the lungs. From these nodules *S. pullorum* was isolated in pure culture. The presence of unabsorbed yolk was a constant feature and in some cases it was adherent to the peritoneum.

MICROSCOPIC PATHOLOGY.

Specimen 6324.—*Liver*: A large number of irregular round foci of various sizes are disseminated throughout the parenchyma. In the Haemalum-eosin stained sections they have a bright pink colour. The smallest consists of one isolated liver cell which appears to have undergone kariolysis and whose cytoplasm has turned to a more or less homogeneous pink staining mass, somewhat resembling hyaline degeneration. This degeneration process can be easily followed. The nucleus first becomes fragmented and the cytoplasm loses its normal staining properties, becomes amorphous, and stains a greyish-pink. Later the chromatin becomes spread in the cytoplasm in dust-like particles, and finally disappears altogether when the homogeneous stage is reached. The larger foci are formed in the same way by degeneration of adjacent cells. Such foci are rarely uniformly hyaline in appearance, as the central portion becomes infiltrated with round cells, while the periphery usually shows blobs of pink homogeneous newly degenerated liver cells. The liver as a whole is hyperaemic. Numerous clumps of bacteria are seen, but, strangely enough, are not closely associated with the necrotic foci. There is also practically a total absence of the eosinophilic polymorphic leucocytes seen in cases of fowl typhoid.

PATHOGENICITY.

Six 4-day-old chickens were each given (per os) .5 c.c. of a 24-hour broth culture. One died on the eighth day and the organism was recovered from the internal organs, viz., liver, spleen, and heart blood.

Four guinea-pigs were injected; two received 1 c.c. each of broth culture subcutaneously, and two 1 c.c. intraperitoneally. Those which were injected intraperitoneally died, but the others showed no untoward effects as the result of the injection.

A similar number of rabbits were injected. Those done by the intraperitoneal route died within four days, and those injected subcutaneously died on the eighth and ninth days respectively. The organism was recovered from the heart blood in these cases.

BACTERIOLOGY.

Morphology.—Small slender gram negative rod with rounded ends. It generally appears singly, is non-motile, and varies from 0.3 to 0.5 by 1.5 to 2.5 microns.

Cultural Features.—Agar: On this medium it grows well. Tiny shiny greyish colonies, almost streptococcic in appearance, are seen after 24 hours' cultivation. The colonies measure from 1 to 2 mm. after 48 hours, when they appear to have reached their maximum growth.

Broth: On plain broth there is a light uniform turbidity within 24 hours after inoculation. There is no pellicle and little sediment.

Gelatin: After 48 hours at room temperature, greyish white colonies are seen. In a stab culture there is a delicate granular growth along the needle track and on liquefaction.

Potato: There is a poor growth on this medium in the form of a shiny streak along the inoculated surface.

Litmus Milk: After 24 hours' inoculation, there is slight acidity, which remains unchanged for about four weeks, then turns slight alkaline again.

Milk is not coagulated.

Indol is not formed.

Lead Acetate Agar: Slight brownish decolouration is noted after 48 hours' inoculation, but not so marked as shown by other strains Nitrates are not reduced.

CARBOHYDRATE REACTIONS.

This medium consists of distilled water containing 2 per cent. peptone, 1 per cent. Andrade's indicator, and 1 per cent. of the test substance. After 15 days' observation in the incubator, galactose, glucose, mannite, sorbite, laevulose, mannose, and rhamnose were fermented without gas production. The latter was not always fermented within this period. Arabinose, lactose, nutrose, inosite, salicin, raffinose, inulin, erythrite, dextrin, saccharose, nutrose, glycerine, maltose, and dulcite were not attacked. Xylose was fermented slightly, if at all, before the 15th day.

This strain has been fairly constant in its biological features. It has been subcultivated for two years and has not shown any transition to gas production, a phenomenon noted by several workers.

Thus far, all the strains isolated in the Transvaal are anaerogenic. Among chickens, the first outbreak in the Union was due to an anaerogenic strain. This is of interest as it is generally held that the aerogenous types of *S. pullorum* are found mostly in chickens and the anaerogenic forms in adult poultry. The type found in Algiers apparently affected both chickens and adult poultry. In its morphological and staining reactions the local strains of *S. pullorum* correspond with *S. gallinarum*, but during primary isolation grow less luxuriantly on artificial media.

Biochemically the described strain corresponds with two more strains isolated in South Africa, except that it does not ferment arabinose.

Local strains of *S. pullorum* differ from *S. gallinarum* in that they fail to ferment maltose, glycerol, and dulcite. The last named is a reliable and stable differential sugar. On litmus milk the acid alkaline transitional period of *S. pullorum* is much longer than that of *S. gallinarum*.

DISTRIBUTION IN SOUTH AFRICA.

The extent of distribution is still under investigation. Besides the outbreak under discussion, on two occasions *S. pullorum* was isolated from diseased ovaries of hens sent to the laboratory, and once in a case from the Orange Free State, on examination of chickens dead in the shells of artificially incubated eggs.

Mr. Mitchell and Mr. Alexander have found the disease to be on the increase in Natal, and it is certain that the spread of bacillary white diarrhoea will have far-reaching effects in retarding the progress of poultry culture in South Africa. It is, therefore, of primary importance that more data of the distribution in the Union be obtained by bacteriological examination, as well as serological testing.

DIAGNOSIS.

A definite diagnosis can only be made in the laboratory. A provisional diagnosis may be made in the field on account of the early appearance of the affection, high mortality, white scour, and the presence of unabsorbed youk in the abdominal cavity.

DIFFERENTIAL DIAGNOSIS.

Not all cases of white diarrhoea in the Union are due to *S. pullorum* infection. Chills and improper feeding sometimes give rise to similar clinical symptoms. Aspergillosis also shows the presence of white diarrhoea, especially when the disease is well advanced. Further, coccidiosis sometimes simulates infectious white diarrhoea, but symptoms do not appear so soon after hatching.

PREVENTION.

The most essential point in the control of bacillary white diarrhoea is to avoid the introduction of diseased chicks and adult chronic carriers. This applies to both the individual poultry owner and the State. The prompt detection and destruction of chronic carriers of the malady is of the utmost importance.

At present serological tests will not differentiate a carrier of *S. pullorum* from one of *S. gallinarum*, a fact which is not of much practical importance in view of the danger of both diseases. Poultry keepers should be thoroughly instructed as to the danger of bacillary white diarrhoea when once introduced in to their flocks. Sellers of day-old chicks should be possessed of a Government or Municipal Certificate, testifying that their flocks are free from bacillary white diarrhoea. Often the disease is brought into a healthy flock through the introduction of a single fowl. It can be avoided by having such a fowl tested before purchasing. Bacillary white diarrhoea should be made a notifiable disease in South Africa before it is too late.

CURATIVE TREATMENT.

Treatment is not recommended in an outbreak of bacillary white diarrhoea, as the surviving chicks may be reservoirs of infection in adult life.

FOWL TYPHOID.

THE earliest references to epizootic typhoid diseases of fowls are to be found in the works of Lemaistre (1869) and Perroncito (1877-1878). Klein (1899) described a serious disease among poultry in Orpington, Kent, England, under the name of infectious enteritis. He isolated the causal organism and named it *Bacillus gallinarum*. This disease is still known on the Continent as Klein's disease. Smith (1894) found a similar disease on Black Island, R.I. Moore (1895) studied a poultry disease in Virginia, which he described under the name of infectious leukemia. He gave a good description of the organism and designated it *Bacterium sanguinarium*.

Curtice (1902) investigated a poultry epizootic in Rhode Island and called the condition "fowl typhoid." Trincas (1908) described a poultry disease in Italy simulating cholera clinically and pathologically, but not bacteriologically. To one of the organisms found he assigned the name *Bacillus pseudo-cholerae*.

Pfeiler and Rehse (1913) studied a fowl typhoid outbreak in Germany and named the organism isolated by them *Bacillus typhi gallinarum alcalifaciens*.

Hadley, Elkins, and Caldwell (1918) have since shown, in their extensive study on the "Colon-Typhoid Intermediates of Birds," that the organisms isolated and described by Moore and Klein respectively are identical. There is little doubt to-day that all the poultry epizootics mentioned above were due to similar organisms. The generally accepted name, however, by priority is *Salmonella gallinarum*, Bergey (1925), although it must be stated that *Salmonella gallinarum* seems to occupy an intermediate position between the *Salmonella* and the *Eberthella* genera, and has biological characteristics in common with both genera.

Outbreaks other than those mentioned have been recorded by Taylor in California (1915), Pfeiler and Roepke in Germany (1917), Van Straaten and Te Hennepe in Holland (1918), Te Hennepe in Holland (1923), Truche in France (1923), Donatien, Plantureux, and Lestoquard in Algiers (1924), and Martinaglia in South Africa (1926). Smith and Ten Broeck (1915), Mulsow (1919), have also made extensive comparative studies of the organism.

In recent years fowl typhoid has received almost as much attention as fowl cholera. As a rule, it is not as virulent a malady as fowl cholera, but the fact that it can easily be disseminated by means of carriers at once renders it a dangerous disease, and from a State veterinary point of view a most important one, as fowls are still introduced into South Africa without being tested to see whether they are carriers of the poultry *Salmonella* or not.

Formerly in South Africa epizootics of poultry were attributed to fowl cholera, especially by poultry owners, and it was not until 1924 that fowl typhoid was also recognized, when it was definitely established both bacteriologically and serologically by the writer. The first outbreaks studied occurred near Pretoria and in Johannesburg. Soon afterwards the disease was diagnosed in all Provinces of the Union. To-day it may be assumed, from experience gained during the last five years, that fowl typhoid is the commonest and most destructive bacterial disease of poultry in the Union.

Numerous fowl typhoid epizootics have been diagnosed all over South Africa, but not a single case of fowl cholera has been established bacteriologically. Regarding fowl typhoid, it is not known when and how the disease was introduced into South Africa. The mortality is generally high, varying from 15 to 80 per cent. of the flock. It sometimes assumes such a virulent form that it almost resembles a fowl cholera outbreak.

In the Union it affects mainly birds from a year upwards. Natural outbreaks so far have been confined to the common fowl, but on two occasions we have been informed by owners, in whose flocks the disease has been diagnosed, that their turkeys were also dying, apparently from the same affection. Recently a bacteriological diagnosis has been made in the case of a turkey sent to the Laboratory.

The organisms can be isolated in pure culture from the bloodstream and various internal organs, especially liver, spleen, and kidneys. They are never plentiful in a stained blood-smear and sometimes show a slight marginal staining. *S. gallinarum* is with very little difficulty obtained from the subclavian vein (of a sick fowl showing a temperature reaction) by drawing blood with a pipette and transferring it to broth.

SYMPTOMS.

In South Africa the disease often takes a virulent form. Some owners may lose almost their entire flocks within a month, while others lose several a day over a long period.

The incubation period under experimental conditions varies from two to six days before the initial rise in temperature is noticed. The comb is often cyanotic in appearance, apparently due to cardiac deficiency, but sometimes the comb and especially the wattles may be pale. Microscopic examination of the blood often shows decrease of the erythrocytes and an increase of the polymorphic eosinophiles.

The temperature varies from 107.5° to 111.5° F. The sick bird shows diminished appetite, increased thirst, and general dulness. The feathers are often ruffled, especially round the neck, and the head is held close to the body. The wings often droop, while the sick fowl stands with closed eyes and has a sleepy appearance. Others may lie down and show disinclination to walk. Regurgitating movements are sometimes observed and a slimy catarrhal condition of the buccal cavity is common. Diarrhoea is a usual feature, the droppings being of a greenish-yellow colour and the feathers around the vent are generally tinted with greenish soft faeces. Just before death the fowl becomes prostrated and the temperature subnormal.

POST-MORTEM APPEARANCES.

Rigor mortis sets in soon after death. On opening the abdominal cavity, yellow or blood-stained fluid is frequently seen. The liver is the most constantly affected organ, being much enlarged, and the surface displaying irregular haemorrhages and yellow patches. In some cases it has a characteristic bronze-like tint. In certain cases the bile ducts are distended and icterus is noticed. If the disease is of more than a week's duration, minute multiple necrotic foci, discrete and sometimes numerous, are often seen in the liver and throughout the parenchyma. The liver, on incision, is soft and friable in consistence. The spleen, as a rule, is enlarged and congested, showing sometimes tiny grey specks under the capsule. It bulges on section, the pulp is dark red and the splenic corpuscles often prominent. The kidneys appear slightly swollen, of a yellowish brown colour and sometimes soft in consistence.

The ovaries and oviduct are often highly inflamed. In laying hens it is not unusual to find egg yolk in the peritoneal cavity. On several occasions *S. gallinarum* was isolated from the yolk of the inflamed ova of fowl typhoid cases. In experimental chronic cases the ovarian lesions, i.e. shrunken ova, are similar to those seen in natural *S. pullorum* carriers. The lungs are usually normal, but when there is fluid in the cavities, they are sometimes grey and oedematous. The pericardial sac often contains a sero-fibrinous exudate. Occasionally grey necrotic areas are seen on the epicardium of the heart. These may take the form of greyish-yellow protuberances where the disease is taking a chronic course. In many cases the heart appears normal. Haemorrhages on the heart have been observed in four out of thirty typhoid epizootics investigated. In these four cases the disease had taken an acute and virulent form. As a rule, the intestines are pale, but a catarrhal condition generally prevails, with isolated haemorrhagic patches on the mucous membrane. In some cases the faecal contents are generally bile-stained and the cloaca, as a rule, contains soft green faecal matter.

MICROSCOPIC PATHOLOGY.

Specimen 5681.—*Liver*: The liver tissue contains a large quantity of blood, distending the intercellular spaces, so that the liver cell columns are spread out. There is a slight amount of vacuolization or fatty degeneration. On looking at the section closely, one notices isolated, or minute groups of liver cells, of which the cytoplasm is hyaline in appearance. These cells are scattered rather uniformly through the parenchyma. They are very often associated with a few round and polymorphic cells, especially where they tend to form small foci. This hyalinization of the cytoplasm does not as a rule affect the nucleus, which may, however, assume a pyknotic appearance. The necrotic process in this case is confined to individual scattered liver cells, instead of being localized to a group of cells forming disseminated foci.

Specimen 4815.—*Liver*: The parenchyma is markedly hyperaemic. The interlobular tissue presents fairly extensive lymphoid accumulations. Fatty degeneration is present to a slight extent. Occasionally, lighter staining centres are seen. These consist of a group of liver cells, in which the cytoplasm has become hyaline in appearance. Such centres are infiltrated by a small number of round cells and polymorphic eosinophiles. Apart from these foci, numerous hyalinized liver cells, occurring singly or in groups of three or four, are scattered throughout the liver tissue. They are usually associated with a few round and polymorphic leucocytes. The parenchyma as a whole shows a diffuse interstitial infiltration with round and polymorphic leucocytes.

Specimen 4793.—*Liver*: Numerous fairly large and diffuse foci are scattered throughout the parenchyma. They are differentiated from the liver structure by the absence of the typical liver cell columns, thus giving them a finely punctiform appearance under low magnification. Their sizes vary and may reach up to about a sixth of the liver lobule. Under high magnification these foci present the following appearance: The liver tissue, over a fairly wide area, is infiltrated with round cells. These increase in number towards the centre, while the liver cell columns become indistinct and disappear altogether. The central part contains numerous polymorphic eosinophiles with rod-shaped granules, and a fair number of erythrocytes. Although the liver cells cánnot be distinguished. their nuclei are still fairly well preserved. The picture is, therefore, more of the nature of a diffuse focal parenchymatous hepatitis than that of a sharply circumscribed necrotic focus. The intralobular tissue shows a fair amount of lymphoid accumulations. The parenchyma shows a noticeable hyperaemia. A feature in many sections examined is the perivascular infiltrations of round cells or polymorphic eosinophiles. The latter contain round or rod-shaped granules.

Spleen: This organ shows very indefinite lesions. In the more acute cases all that can be noted is more or less intense hyperaemia. Later there appears a progressive hyalinization of the tissue around the pencil arteries. Similar lesions have also been encountered in the spleen of fowls in spirochaetosis. Well-defined necrotic foci are sometimes seen, but cannot be regarded as constant features.

Heart: Sometimes well-defined tuberous yellowish-grey nodules are found on the heart of chronic cases of fowl typhoid. These may produce lesions of chronic myopericarditis. The miscroscopic pathology of these cases is interesting. They appear to be leucotic in nature. These elevations consist of an accumulation of cells of the round cell and polymorphic-eosinophilic type. There are also signs of vascularization and organization. The myocardium is infiltrated with round cells: these cause pressure atrophy of the muscle fibres and progressive displacement. The picture is that of a leucosis.

De Kock (1928) points out the present unsatisfactory state of our knowledge regarding the leucosis of fowls, and its relation to the various round cell or polymorphic hyperphasias often met with.

BACTERIOLOGY.

The strains isolated and studied in the Union conform more or less to the latest American and European descriptions of the *S. gallinarum* type. They may briefly be described as irregular non-motile rods with rounded ends, measuring 0.3 to 0.6 by .2 to 0.2μ. They stain well with all the laboratory dyes in common use, and are gram negative. They sometimes show a marginal staining with a light centre. Agar colonies often show a yellow centre when freshly isolated. They are moist and circular with entire margin. Gelatin colonies are delicate and circular with finely granular appearance. On broth there is uniform cloudiness with slight flocculent sediment. Gelatin is not liquefied, milk not coagulated and indol not formed. Litmus milk is turned acid within 24 hours, and becomes slowly alkaline from the third day onwards. Lead acetate agar is blackened along the agar track within 48 hours. A carbohydrate medium, consisting of distilled water containing 2 per cent. peptone, 1 per cent. Andrade's indicator, and 1 per cent. of the test substance, and incubated for 15 days, gave the following results: Arabinose, rhamnose, galactose, klucose, mannite, sorbite, maltose, xylose, dulcite, laevulose, mannose, and glycerol were all fermented without gas-production. Saccharose, dextrine, erythrite, inulin, raffinose, salicin, inosite, nutrose and lactose were not attacked.

COMMENTS ON A FEW SUGARS.

Rhamnose.—The local strains ferment this important differential sugar of the *Salmonella* and *Eberthella* genera. It is attacked between the 3rd and 14th days, but one or two strains are only attacked between the 3rd and 4th weeks.

Sorbite.—This sugar is slowly fermented, some strains just before the 14th day and others afterwards. The few local human typhoid strains in our possession ferment this sugar quickly.

Xylose.—This sugar is weakly fermented by our *gallinarum* strains.

Dextrin.—Fermentation tests with this sugar have been unreliable.

THE FOWL TYPHOID CARRIER.

Although much has been written about the transmission of *Salmonella pullorum* by carriers, comparatively little is known about the natural history of *S. gallinarum* infections. Often it is difficult to explain the sudden occurrence of epizootics, especially where there is no record of recently introduced fowls. Several times sparrows have been suspected as carriers where fowl typhoid epizootics occurred, but this may be coincidental. As yet no experimental evidence has been brought forward to incriminate the sparrow as a carrier of fowl typhoid in South Africa.

Undoubtedly the disease is spreading and its distribution is far and wide throughout the Union. Once having made its appearance in any locality, it recurs from time to time at longer or shorter intervals, unless drastic sanitary measures are adopted.

Beaudette (1925) having isolated *S. gallinarum* from the heart blood and unabsorbed yolk of diseased chickens and, in one instance, from a pathological ovary of an adult hen, recorded the possible transmission of fowl typhoid through the egg. Kaupp and Dearstyne (1924) referred to a fowl which recovered from typhoid. This bird had a relapse later, and the writers emphasize the danger of such a bird when purchased.

While working on fowl typhoid and attempting transmission experiments per os, an opportunity was afforded of keeping under observation fowls which had survived the experiments. One experiment comprised ten fowls in one camp, each having received 1 c.c. of a 24-hour broth culture in its second generation. Four died showing typical lesions and the others recovered. In one hen, which was killed one month afterwards, no lesions were noticed except that the gall-bladder appeared atrophied. It was punctured by means of a sterile pipette, and the golden-coloured bile transferred to a small tube of broth and again to an agar slant. The following day 33 fowl typhoid-like colonies were noticed on the slant. Several colonies subjected to a thorough bacteriological and serological examination proved to be typical *S. gallinarum*, similar to the strain which was used for infecting the fowl. Three months after the time of infection the rest of the fowls were all killed and bacteriologically examined. Three hens showed no conspicuous lesions, except an inflammation of the ovaries in one instance. Cultures were made from the various organs, including the ovaries and gall-bladder, but no organisms were cultivated. One large cock, however, showed a marked fibrinous pericarditis, with several cysts on the auricle between the large vessels and one large bulging tumour-like protuberance, measuring about 1 c.c. in diameter. (See Fig. 24.) Cultures were made from these heart lesions and the next day a culture of *S. gallinarum* was growing in the broth which was inoculated from the cysts. The agar slant inoculated from the greyish-yellow protuberance on the heart displayed twelve typical fowl typhoid-like colonies. These were subsequently identified as *S. gallinarum*. One hen had diseased ovaries, showing several shrunken pedunculated ova. The capsule was thick, containing yellow-brown yolk, and cultures made from the contents of these ova gave a rich growth of *S. gallinarum*. (See Fig. 25.)

In a second experiment, eight fowls also received per os 1 c.c. each of a 24-hour culture of another strain of *S. gallinarum* in its second generation. Four of these died from the disease and four survived after a severe illness. The sera of three of these were tested one month later with *S. gallinarum*, several other species of *Salmonella*, and one of *Eberthella*. Their agglutination titre is shown in Table No. IV. Six months after infection their sera were again tested against *S. gallinarum*, and were still found positive as shown in the table referred to. Finally, these three fowls were killed for bacteriological examination. The Leghorn showed large old yolk concretions in the abdominal cavity and a diseased oviduct. Cultures from these concretions and oviduct yielded a rich coliform growth, but no organisms of *Salmonella* type were isolated. The Plymouth Rock hen on necropsy showed a number of old abdominal yolk adhesions and diseased pedunculated ova. These yolk adhesions contained a brown material and fat droplets. Cultures from these, including some of the diseased ova, gave a rich growth of *S. gallinarum*. The faeces in these cases was not examined. Three months after these fowls were infected, they were placed in contact with four Leghorn hens and one cock. The sera of these birds were examined before placing in contact and found negative to the agglutination test. After having been three months in contact, their sera were examined again and it was found that one hen gave a strong agglutination titre of 1 in 6,000, another 1 in 500, and the rest negative. These fowls have not been killed, as it is intended to use them again.

The fact that fowls can show a high agglutination titre six months after showing symptoms and continue to harbour *S.*

gallinarum in pathological conditions is significant. It will be noted, further, that the two in-contacts also reacted to the agglutination test without showing clinical symptoms. Although these findings are not conclusive, there is little doubt that the fowl may be a potential carrier of fowl typhoid organisms from one country to another.

PATHOGENICITY.

Fowls.—The organisms are pathogenic for fowls when fed per os, but some strains are more virulent than others. On subcultivation these organisms soon lose their virulence.

Guinea-pigs are susceptible to some strains and refractory to others. Rabbits are more susceptible than guinea-pigs, especially when injected intraperitoneally. Two sheep receiving each 1 c.c. of a 24-hour broth culture subcutaneously showed no ill-effects.

SEROLOGY.

The agglutination titre of fowls which contracted the disease in nature is often very high and may go up to 6,000 and even higher. The agglutination results of various *Salmonella* strains and local *S. gallinarum* strains are shown in Tables Nos. V and VI.

Organisms not agglutinated by *S. gallinarum* sera (S.A.) are *S. paratyphi*, *S. schottmulleri*, three *S. aertrycke* strains in our possession and *S. suipestifer*, *B. paratyphosis* C. (Hirschfeld), and *B. typhi murium.* The last-named organism appears to be an *aertrycke* and it has been assigned an unfortunate name. The association of such a cosmopolitan type with the name of the host from which it was isolated is objectionable in describing species, as it will lead either to hair-splitting or to confusion.

TABLE No. IV.

Sera of three fowls infected with a South African strain of S. gallinarum *tested against* S. gallinarum *and other* Salmonella *strains one month after infection. (One Eberthella.)*

Fowl Tested.	Bacterial Emulsions.	Bacterial Dilutions.								
		$\frac{1}{25}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$
Leghorn Hen.............	*E. typhi* (human)...............	+++	+++	+	−	−	−	−	−	−
	S. gallinarum (S.A.)............	+++	+++	+++	+++	+++	+	−	−	−
	S. enteritidis 52................	+++	+++	+	−	−	−	−	−	−
	S. pullorum (S.A.)..............	+++	+++	+++	+++	+++	+	−	−	−
	S. abortivo-equinus (S.A.)........	+++	+++	+++	+++	+++	+	−	−	−
	S. aertrycke (Jordan, Am.).......	+	−	−	−	−	−	−	−	−
Plymouth Rock...........	*E. typhi* (human)...............	+++	+++	+++	+++	+++	+	−	−	−
	S. gallinarum (S.A.)............	+++	+++	+++	+++	+++	+++	+	−	−

Black Orpington	*S. enteritidis* 52	+++	+++	+++	+++	+++	+	−	−	−
	S. pullorum (S.A.)	+++	+++	+++	+++	+++	+++	+	−	−
	S. abortivo-equinus (S.A.)	+++	+++	+++	+++	+++	+	−	−	−
	S. aertrycke (Jordan, Am.)	+	−	−	−	−	−	−	−	−
	E. typhi (human)	+++	+++	+++	+	−	−	−	−	−
	S. gallinarum (S.A.)	+++	+++	+++	+++	+++	+	−	−	−
	S. enteritidis 52	+++	+++	+++	++	+	−	−	−	−
	S. pullorum (S.A.)	+++	+++	+++	+	−	−	−	−	−
	S. abortivo-equinus (S.A.)	+++	+++	+++	+++	++	+	−	−	−
	S. aertrycke (Jordan, Am.)	+	−	−	−	−	−	−	−	−

Six months after infection the sera of the birds mentioned above were tested again with the following results:—

Leghorn Hen: 1 in 800.
Plymouth Rock: 1 in 1,600.
Black Orpington: 1 in 200.

TABLE No. V.

AGGLUTINATION TEST.

Serum of sheep immunized against a South African strain of S. gallinarum *tested against different* Salmonella *and one* Eberthella *species.*

Origin of Strain.	*Salmonella* Emulsions.	Bacterial Dilutions.								
		$\frac{1}{25}$	$\frac{1}{50}$	$\frac{1}{100}$	$\frac{1}{200}$	$\frac{1}{400}$	$\frac{1}{800}$	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$
Lister Institute	*B. enteritidis* (Gärtner)	+++	+++	+++	+++	++	–	–	–	–
America	*B. aertrycke* (Jordan)	–	–	–	–	–	–	–	–	–
Natal	*S. aertrycke* (canary)	–	–	–	–	–	–	–	–	–
Transvaal	*S. aertrycke* (calf)	–	–	–	–	–	–	–	–	–
America	*B. abortivo-equinus*	+++	+++	+++	+++	–	–	–	–	–
Transvaal	*E. typhi* (human)	+++	+++	+++	+++	+++	+	–	–	–
Transvaal	*S. abortivo-equinus*	+++	+++	+++	++	–	–	–	–	–

Transvaal	*S. gallinarum*	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	−
Lister Institute	*B. typhi murium*	−	−	−	−	−	−	−	−	−
Transvaal	*S. gallinarum*	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−	−
Natal	*S. pullorum*	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	++	−	−
Lister Institute	*B. paratyphosis* C. (Hirschfeld)	−	−	−	−	−	−	−	−	−
Lister Institute	*B. paratyphi* A.	+	−	−	−	−	−	−	−	−
Lister Institute	*B. paratyphosis* B. (*Schottmulleri*)	−	−	−	−	−	−	−	−	−
Transvaal	*S. enteritidis* (calf)	+ + +	+ + +	+ + +	+ + +	+	−	−	−	−
Lister Institute	*B. paracoli* (Jensen)	+ + +	+ + +	+ + +	−	−	−	−	−	−
Lister Institute	*B. gallinarum* (Klein)	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−	−
America	*B. suipestifer*	−	−	−	−	−	−	−	−	−
South Africa	*S. pullorum*	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−
Lister Institute	*B. pullorum*	−	−	−	−	−	−	−	−	−
Lister Institute	*B. pullorum*	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−
Lister Institute	*B. pullorum*	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	−	−
Lister institute	*B. sanguinarium* (Harvard)	+ + +	+ + +	+ + +	+ + +	+ + +	+	−	−	−

TABLE No. VI.

AGGLUTINATION TEST.

Serum of sheep immunized against a South African strain of S. gallinarum *tested against* S. gallinarum *strains enumerated below.*

Origin of Strain.	Serial Nos. of Epizootics.	Bacterial Dilutions.								
		1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400
Transvaal	1	+++	+++	+++	+++	+++	+++	++	+	—
Free State	2	+++	+++	+++	+++	+++	+++	+++	—	—
Cape Province	3	+++	+++	+++	+++	+++	+++	+++	—	—
Transvaal	4	+++	+++	+++	+++	+++	+++	+	—	—
Natal	5	+++	+++	+++	+++	+++	+++	—	—	—
Transvaal	6	+++	+++	+++	+++	+++	+++	+++	—	—
Transvaal	7	+++	+++	+++	+++	+++	+++	+++	—	—

Transvaal	8	+++	+++	+++	+++	+++	+++	+	—	—
Free State	9	+++	+++	+++	+++	+++	+++	+++	—	—
Transvaal	10	+++	+++	+++	+++	+++	++	+	—	—
Cape Province	11	+++	+++	+++	+++	+++	+++	+	—	—
Natal	12	+++	+++	+++	+++	+++	++	—	—	—
Free State	13	+++	+++	+++	+++	+++	+++	+	—	—
Transvaal	14	+++	+++	+++	+++	+++	+++	+	—	—
Free State	15	+++	+++	+++	+++	+++	+++	++	—	—
Free State	16	+++	+++	+++	+++	+++	+++	+++	—	—
Transvaal	17	+++	+++	+++	+++	+++	+++	++	—	—
Lister Institute, England, *B. gallinarum* (Klein)	—	+++	+++	+++	+++	+++	+	—	—	—
Lister Institute, *B. sanguinarium* (Harv.)	—	+++	+++	+++	+++	++	—	—	—	—
Negative Controls	1	—	—	—	—	—	—	—	—	—
	2	—	—	—	—	—	—	—	—	—
	3	—	—	—	—	—	—	—	—	—

DIFFERENTIAL DIAGNOSIS.

Fowl typhoid should be differentiated from spirochaetosis, leucaemia, fowl cholera, coccidiosis, arsenical poisoning, and a condition of heavy layers associated with ovarian disturbances and white deposit on the pericardium and visceral organs.

DIAGNOSIS.

Diagnosis of fowl typhoid is not always an easy matter and it is only by thorough bacteriological investigation that it can be established beyond doubt. The rapid agglutination test devised by Gwatkin (1923) and modified by Huddleston and Carlson (1926) gives excellent results when sera are submitetd, but will not differentiate between *S. gallinarum* and *S. pullorum* infections. The fowl typhoid organisms generally predominate and can be isolated with comparative ease even where decomposition is advanced. The sending of suitable material, e.g. leg of a suspected case, as advocated by Donatien, Plantureux, and Lestoquard (1924), for bacteriological examination of marrow, is a practical one. Clinically the diagnosis can be provisionally made in South Africa by the high fever, greenish-yellow diarrhoea, and on necropsy, by finding the characteristic lesions.

RECOMMENDATIONS FOR COMBATING THE DISEASE.

As sanitation is of primary importance in controlling epizootics in poultry, the following measures are advised:—

(1) Fowls should be penned off from the free range at the time of the outbreak, in order to diminish the danger of spreading the disease.
(2) All in-contact fowls should be segregated; the sick birds killed and the carcases, with those which have died from the disease, destroyed by burning.
(3) The droppings should be collected daily and burned or sprayed with any of the standard disinfectants.
(4) The walls, floors, and perches of the fowl-house should be sprayed with any efficient disinfectant.
(5) Remove all shade-producing vegetation from the old runs and work in (1 to 2 inches deep) unslaked lime; if possible, allow them to remain unoccupied until after the winter.
(6) Add half teaspoonful of chloride of lime to every four gallons of drinking-water.
(7) All receptacles for food and water should be scalded with boiling water as long as the epizootic rages.
(8) Spreading of the disease by sparrows or other possible vectors should be controlled by the use of suitable netting.
(9) Birds which have been purchased from another breeder should be isolated in a separate pen and removed as far as possible from the fowl-runs for a period of two weeks in order to guard against the introduction of disease. Agglutination tests should be carried out to detect carriers.

Vaccine Treatment.—In the past, fowl typhoid vaccine has been issued to poultry keepers immediately after the disease has been diagnosed, with the request that all healthy fowls be removed to clean ground and vaccinated. Judging from letters received, it would appear that vaccine treatment has given encouraging results. The dose prescribed is 1 c.c. per fowl to be inoculated subcutaneously on the inside of the thigh or under the wing. Apparently better results are to be expected in natural rather than artificial (per os) infections.

SUMMARY AND CONCLUSIONS.

(1) Fowl typhoid is at present the commonest and apparently the most serious bacterial disease of poultry in South Africa.

(2) It often simulates fowl cholera very closely both in virulence and post-mortem lesions, and there may be manifested even haemorrhages on the epicardium in exceptional cases.

(3) Fowl cholera is unknown in the Transvaal and has not been bacteriologically diagnosed elsewhere in the Union.

(4) All breeds are susceptible and those affected are generally in the best of condition. The disease seems to be confined mainly to adult birds.

(5) The disease is a bacteriaemia and the causal organism can always be recovered from the subclavian vein during the fever reaction.

(6) The organism still predominates in the heart blood even after post-mortem changes are already advanced.

(7) Some strains are much more virulent for laboratory animals than others.

(8) The disease was transmitted to fowls on three occasions by feeding freshly isolated cultures. Pigeons fed per os showed no ill-effects.

(9) Fowls which have passed through the disease experimentally harbour *S. gallinarum* for a long period in the following organs: cyst on the auricle, leucosis-like nodule of the ventricle, diseased ovaries, abdominal yolk adhesions, and exceptionally in the gall-bladder. Some fowls which are in contact with such carriers develop agglutinins in their blood. There is thus ample time for *S. gallinarum* to persist in pathological conditions of fowls which have survived an attack of fowl typhoid when imported from abroad.

(10) The sera of South African strains of *S. gallinarum* agglutinate *E. typhi*, *S. pullorum*, *S. abortivo-equinus* isolated locally, but not *S. aertrycke*, *S. suipestifer*, *S. paratyphi*, and *S. schottmulleri*.

(11) Vaccine treatment apparently protects against natural infections.

SALMONELLA AERTRYCKE INFECTION OF CANARIES.

On two occasions, severe epiornithics among canaries have been investigated in the Union, and in each instance a member of the *Salmonella* group was isolated. On further study, the organism was identified as a type of *Salmonella aertrycke*. This is not only of veterinary, but also of medical interest, as this organism is held to be the main cause of food-poisoning in man. In a previous article by Viljoen and Martinaglia (1926). *Salmonella aertrycke* was also incriminated in a case of calf paratyphoid fever. In canaries, diseases attributed to paratyphoid B. organisms were first described by Joest (1906) and later observed by Gilruth (1910), Pfeiler (1911), Adam and Meder (1912), Harkin (1926), who recorded a mortality of 75 per cent., and Gray (1928). Beaudette and Edwards (1925) recorded the first *B. aertrycke* infection of canaries. They investigated an outbreak in New Jersey, where there was a death rate of 35 per cent.

During the summer of 1925 an outbreak of salmonellosis in canaries was diagnosed in Natal by Mr. D. T. Mitchell, M.R.C.V.S., and the writer, the first instance in the Union where this organism was identified as a pathogenic entity of canaries. Since then a case occurring in an aviary on the Rand has been diagnosed by Dr. E. M. Robinson at Onderstepoort. In these cases the owners incurred heavy losses.

On investigation, the main clinical features were: Ruffled feathers, depression, and accelerated breathing. The chief post-mortem appearances were: rigor mortis, enlarged spleen, enteritis, and sometimes hyperaemia of the liver. Blood-smears stained with Giemsa showed the presence of small rod-shaped organisms, but they were not plentiful. The organisms were isolated in pure culture from the heart blood and spleens of dead canaries sent in for autopsy, also from sick ones which were subjected to an overdose of chloroform.

Altogether, three strains of the organisms were studied; these were culturally, biochemically, and serologically identical. Two were isolated from spleens and one from heart blood.

BACTERIOLOGY.

Morphology.—Gram-negative rods with rounded ends. Occasionally coccal or an exceptionally long rod may be seen. They occur singly and are moderately motile, stain well with all the common stains employed in the laboratory, especially carbol-fuchsin.

Cultural Features.—Agar: On plain agar there is abundant growth within 24 hours after inoculation. Single colonies are bluish-grey, smooth shiny, circular, slightly convex, with entire margin. On agar slant there is a filliform confluent rich greyish growth.

Broth: On plain broth there is turbidity within 16 hours after inoculation. There is generally a ring and thin grey pellicle; later, sediment collects at the bottom of the tube.

Gelatine: On plates, colonies appear bluish-grey with undulate margin after a few days at room temperature.

On stab there is progressive spread of surface growth, with moderate filiform growth along the track of the needle and no liquefaction.

Potato: On this medium, growth is not vigorous nor characteristic.

BIOCHEMICAL REACTION.

Litmus Milk.—There is an initial acidity for about 36 hours after inoculation followed by progressive alkalinity.

Milk.—After incubation of a week to 10 days this medium turns slightly yellowish.

Lead acetate agar.—A brownish black line develops along the stab within 24 hours after incubation.

Indol.—Not formed.

Nitrates are reduced to nitrites.

It ferments sodium d. tartrate differential medium of Jordan and Harmon within 24 hours.

Carbohydrate Reactions.

This medium consists of distilled water containing 1 per cent. peptone, Andrade's indicator, and 1 per cent. of the test substance. Saccharose, dextrin, erethrite, inulin, raffinose, salicin, nutrose, and lactose were not attacked.

Arabinose, rhamnose, galactose, glucose, mannite, sorbite, maltose, xylose, dulcite, laevulose, mannose, were fermented with acid and gas.

Inosite was fermented without gas production.

Glycerol slightly fermented without gas production and reduction of the indicator after 48 hours.

SEROLOGICAL IDENTIFICATION.

Two rabbits were immunized with the canary strain at weekly intervals. Each rabbit received four 1 c.c. injections: one intraperitoneal and two intravenous of killed cultures, followed by one intravenous injection of live culture.

The canary anti-serum was tested against typical *Salmonella enteritidis*, *S. aertrycke*, and the canary organism. It failed to agglutinate *S. enteritidis*, but *S. aertrycke* and the canary strain were agglutinated to the titre limit of 1 in 6,400 dilution.

The serological and biochemical tests proved that the organism isolated from canaries belonged to the *aertrycke* group of *Salmonella*.

PATHOGENICITY.

Two adult fowls were each given one broth culture, per os, without apparent ill-effects.

Two rabbits received each ½ c.c. of 24-hour broth culture, the one subcutaneously, the other intraperitoneally. The one which received the intraperitoneal injection died 48 hours afterwards; the other which received the subcutaneous dose died on the fourth day.

Two guinea-pigs which were injected similarly, and with the same dose as the rabbits, also succumbed. The one which was injected intraperitoneally died 36 hours after the operation; the one which received the subcutaneous dose died on the third day.

The animals which received the intraperitoneal injection showed peritonitis, omental suppuration, orchitis, and in the case of the guinea-pig also excessive fluid in the thoracic and abdominal cavities. Cultures were made from the heart blood of these animals during autopsy, and the organisms were recovered again in pure culture.

DISCUSSION.

Salmonella aertrycke is apparently the most cosmopolitan of the *Salmonella* group; the widespread distribution in nature is due to its variety of animal hosts. It is the cause of enteritis in mice, guinea-pigs, parrots, squabs, chickens, and occasionally it is found in pigs, calves, sheep, and man.

The occurrence of disease in an aviary may follow the introduction of a carrier or the contamination of food and water by faeces of avian and rodent vectors.

Salmonella schottmulleri (*B. paratyphosus* B.), as has been shown by Savage and White, is essentially a *Salmonella* of man, and shows much more host specialization than the closely related *S. aertrycke*. The importance of *B. paratyphosus* B. as a cause of disease in animals has been over-emphasized.

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FIGS. 1–6 AND CHARTS 1–4.—CALF PARATYPHOID.

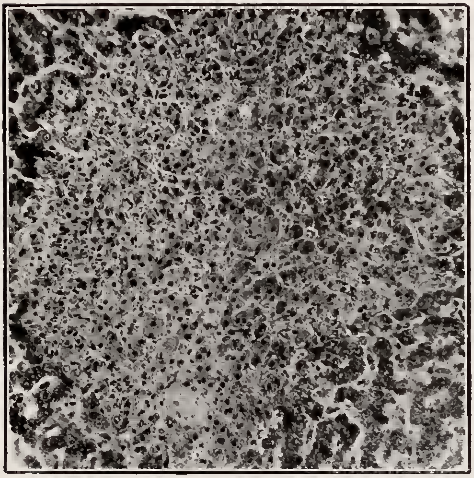


FIG. 1.

Early Calf Paratyphoid lesion, showing focal hyperaemia and beginning of necrosis of liver cells. Note pyknosis of nuclei.

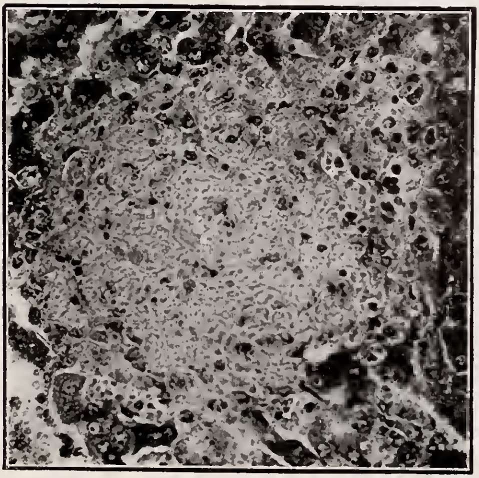


FIG. 2.

More advanced focus, showing local destruction of liver cells with influx of red blood cells.

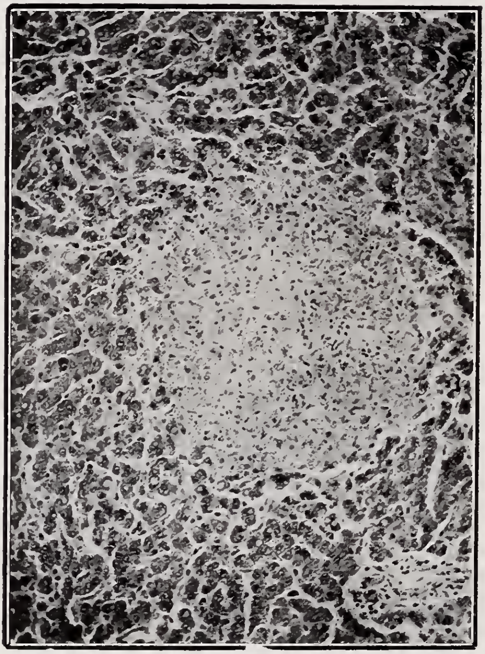


Fig. 3.
Advanced Calf Paratyphoid focus of liver, showing necrobiosis and leucocytic infiltration.

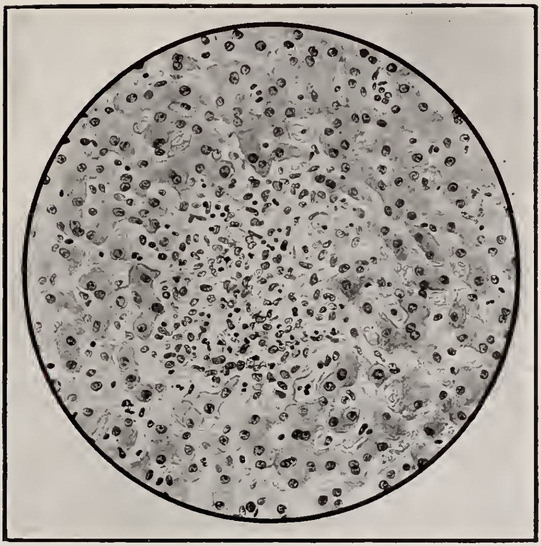


FIG. 4.
Human Typhoid fever focus of the liver. Reproduced from MacCallum's Text-book of Pathology.

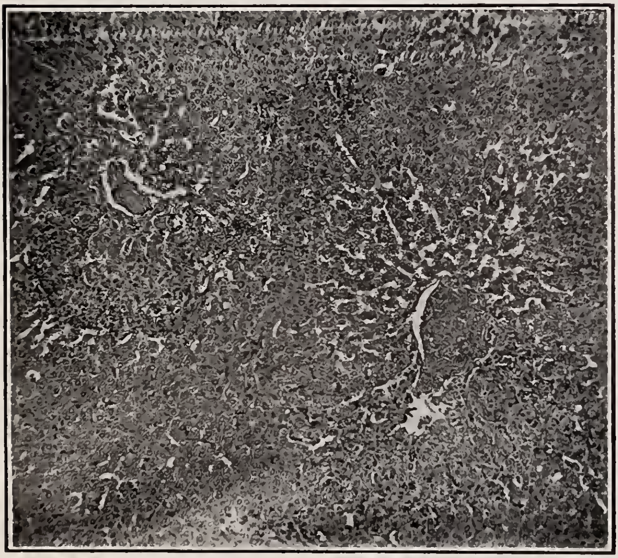


FIG. 5.
Calf Typhoid, showing diffuse acute parenchymatous hepatitis. Note thrombo-phlebitis.

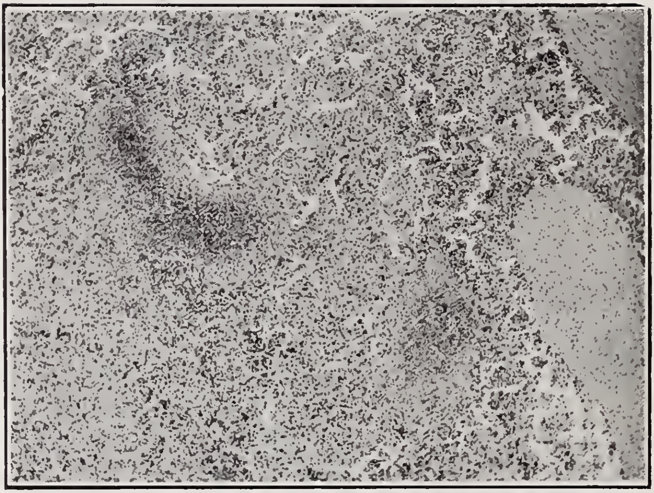
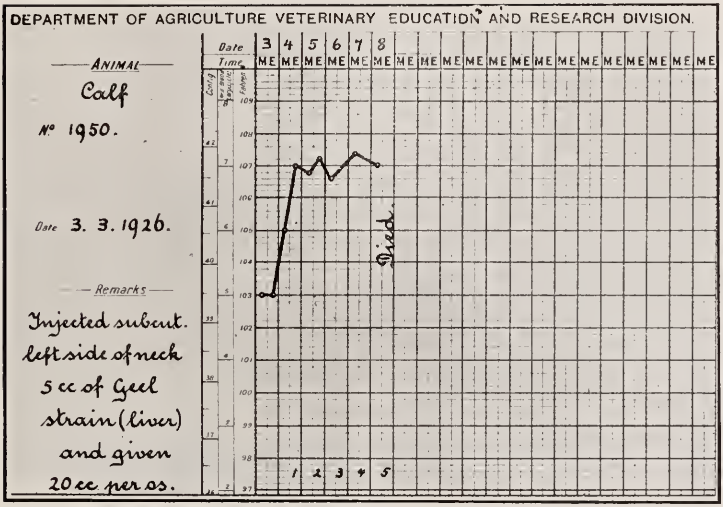


FIG. 6.
Calf Typhoid spleen. Note necrotic foci.



DEPARTMENT OF AGRICULTURE VETERINARY EDUCATION AND RESEARCH DIVISION.

ANIMAL Calf

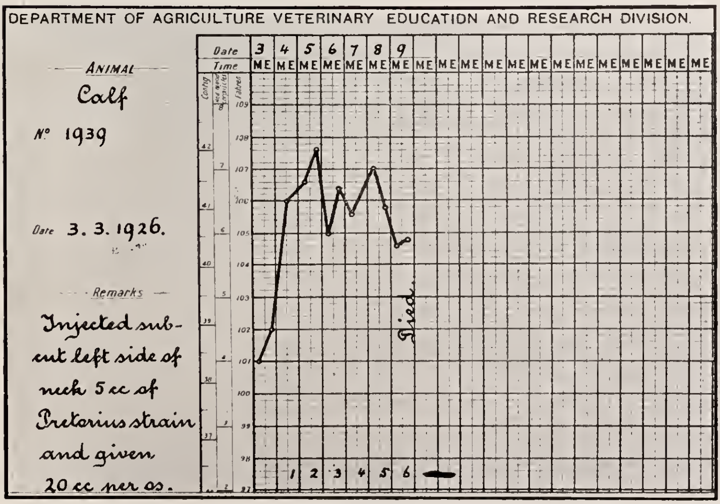
Nº 1950.

Date 3. 3. 1926.

Remarks

Injected subcut. left side of neck 5 cc of Geel strain (liver) and given 20 cc per os.

Chart 1.



DEPARTMENT OF AGRICULTURE VETERINARY EDUCATION AND RESEARCH DIVISION.

ANIMAL Calf

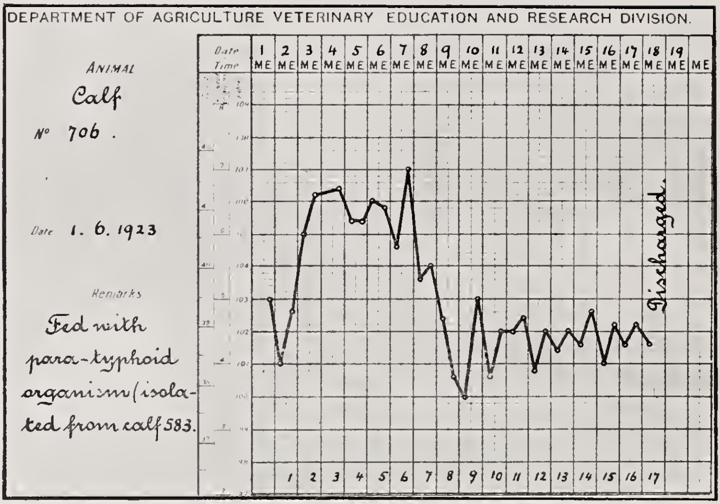
Nº 1939

Date 3. 3. 1926.

Remarks

Injected sub-cut left side of neck 5 cc of Pretorius strain and given 20 cc per os.

Chart 2.


DEPARTMENT OF AGRICULTURE VETERINARY EDUCATION AND RESEARCH DIVISION.

ANIMAL Calf

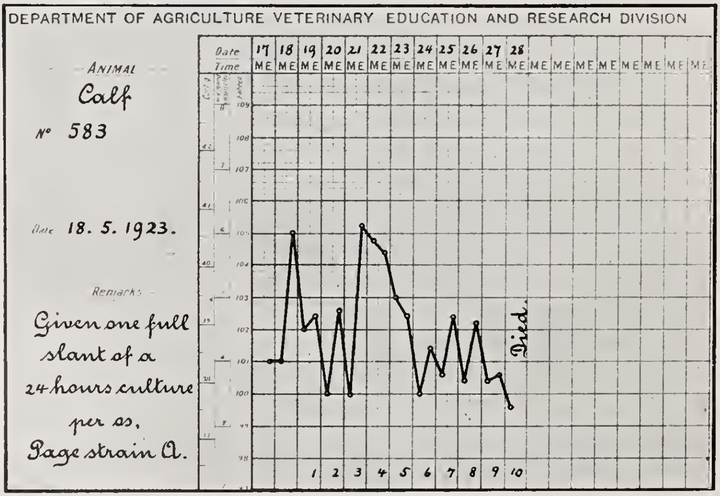
Nº 706.

Date 1. 6. 1923

Remarks

Fed with para-typhoid organism (isolated from calf 583.

CHART 3.


DEPARTMENT OF AGRICULTURE VETERINARY EDUCATION AND RESEARCH DIVISION

ANIMAL Calf

Nº 583

Date 18. 5. 1923.

Remarks

Given one full slant of a 24 hours culture per os. Page strain A.

CHART 4.

FIGS. 7–17.—TENDO-VAGINITIS OF HORSES.

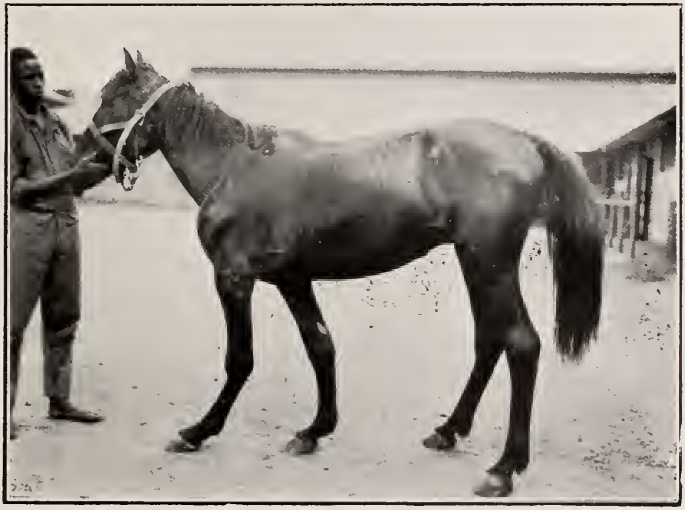


FIG. 7.

Horse No. 15618, showing tendo-vaginitis of post-carpal sheath following Horse-sickness inoculation.

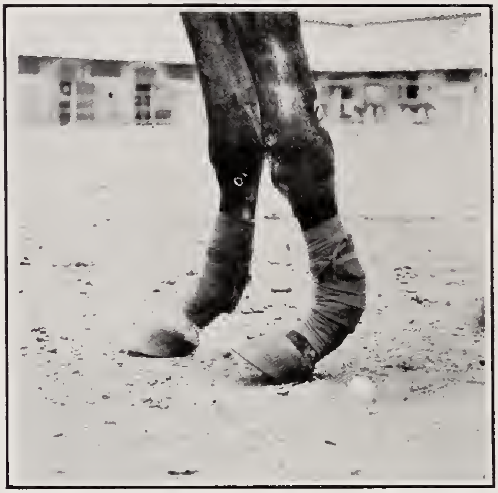


Fig. 8.
Horse No. 15529. Rupture of main tendon of post-carpal sheath; sequent to Horse-sickness immunization.

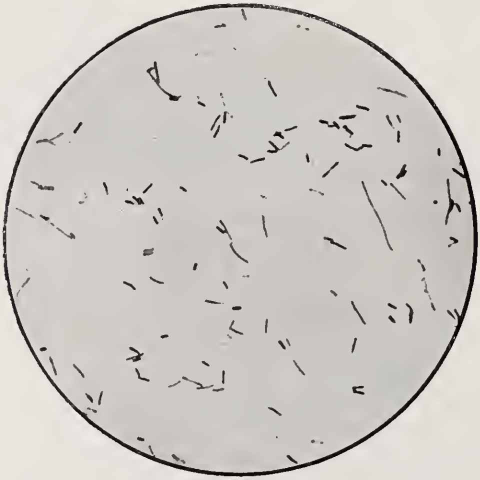


Fig. 9.
Abortivo equinus. Onderstepoort strain 48 hours' agar culture. Carbol fuchsin. × 1000.

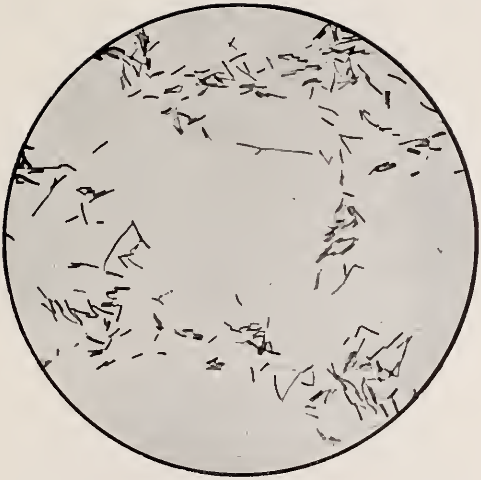


Fig. 10.
S. abortivo equinus. American strain 48 hours' agar culture.
Carbol fuchsin. × 1000.

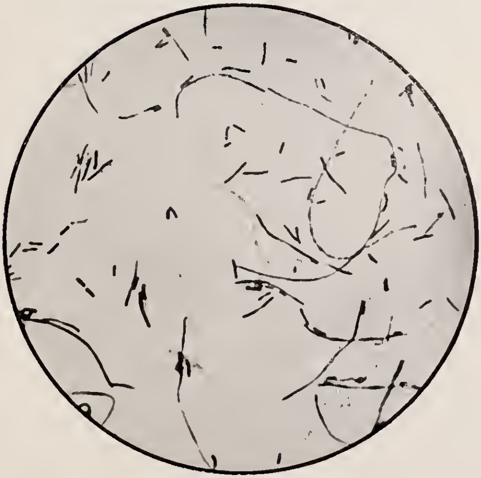


Fig. 11.
S. abortivo equinus. Mule strain, 48 hours' agar culture.
Carbol fuchsin.

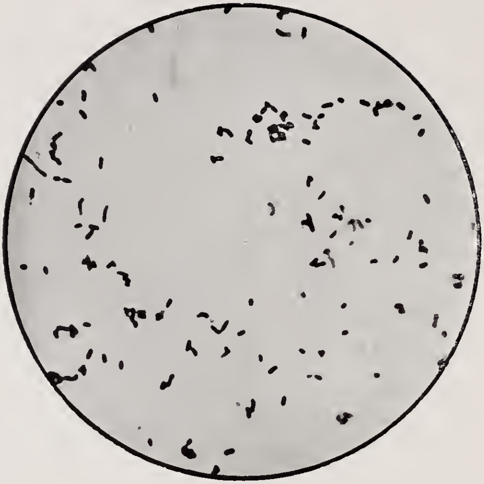


FIG. 12.
S. abortivo equinus, freshly isolated Onderstepoort strain. 36 hours' agar culture. Dilute carbol fuchsin. × 1000.

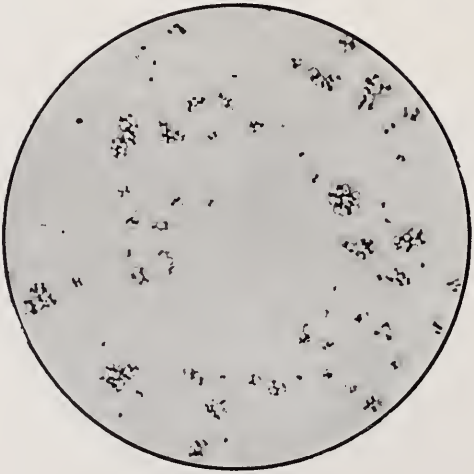


FIG. 13.
S. abortivo equinus, 48 hours' potato culture. Dilute carbol fuchsin. × 1000.

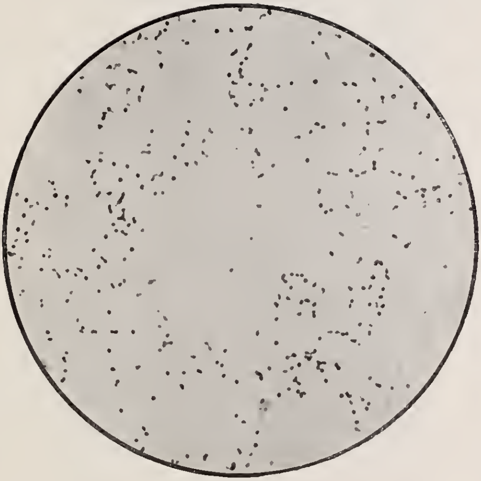


FIG. 14.
S. abortivo equinus, 24 hours' serum agar culture. Ziehl's carbol fuchsin. × 1000.

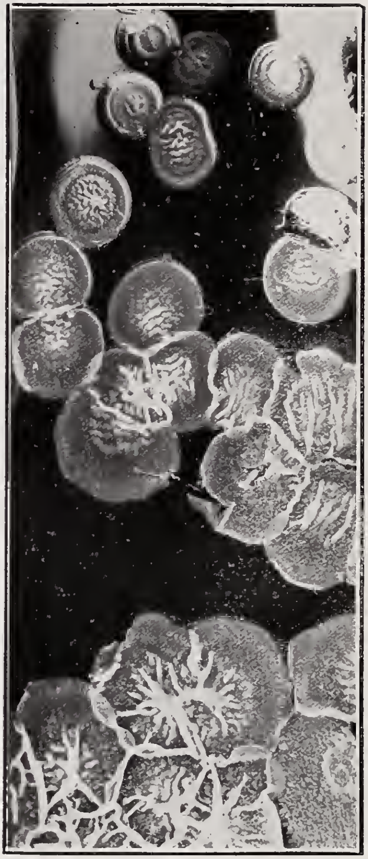


FIG. 15.

S. abortivo equinus, Onderstepoort strain. Six days' old agar culture. × 4. Showing concentric ringed appearance of colonies, and wrinkling near condensation water.

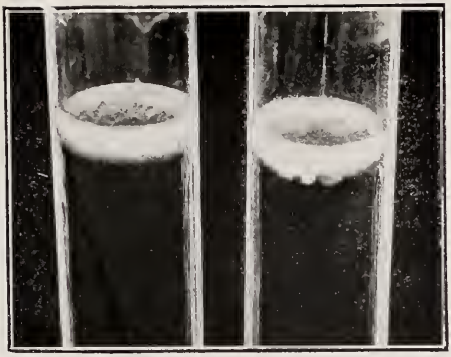


Fig. 16.

Ten days old broth cultures of Onderstepoort horse and mule strains of *S. abortivo equinus*, showing characteristic papery pellicle.

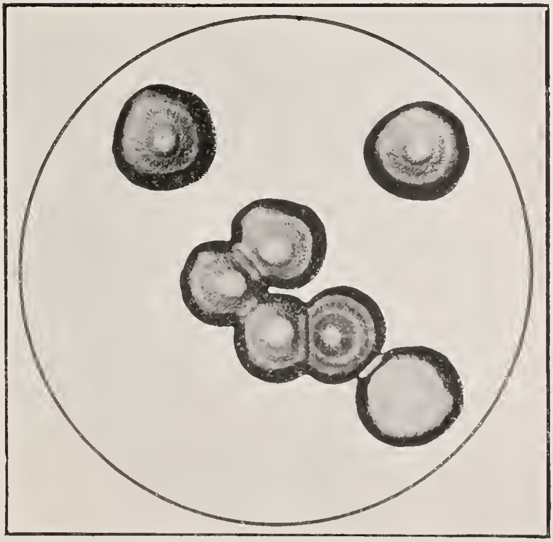


Fig. 17.

On gelatin, four days old thinly seeded colonies.

FIGS. 18–19A.—BACILLARY WHITE DIARRHOEA OF CHICKENS.

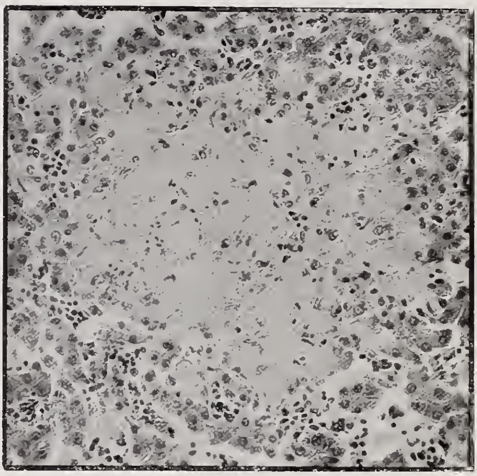


FIG. 18.
Lesion of Bacillary White Diarrhoea in liver of chicken. Necrotic focus.

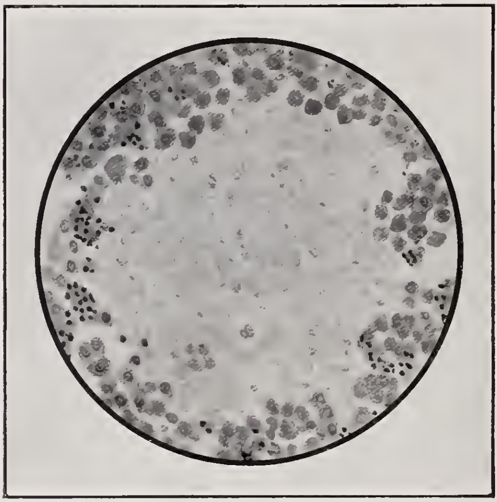


FIG. 19.
From drawing. Same as above.

Agglutination Test for B.W.D.

Serum Dilutions.	1/25	1/50	1/100	1/200	1/400	1/800
Fowl A	+	+	+	+	+	+
,, B	–	–	–	–	–	–
,, C	+	+	+	+	–	–
,, D	–	–	–	–	–	–
,, E	+	+	+	+	+	–
,, F	+	+	+	+	–	–

Negative Controls						
Chick 1	–	–	–	–	–	–
Fowl 2	–	–	–	–	–	–
,, 3	–	–	–	–	–	–

Fig. 19a.

FIGS. 20–32 AND CHARTS 5–6.—FOWL TYPHOID.

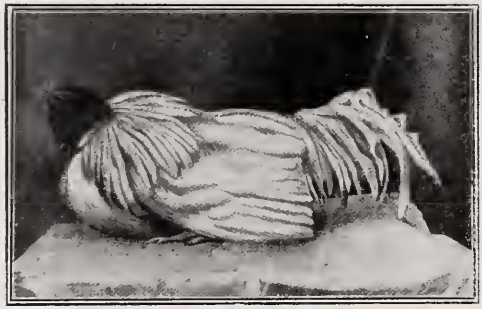


FIG. 20.
Bird suffering from Experimental Fowl Typhoid *in extremis.*

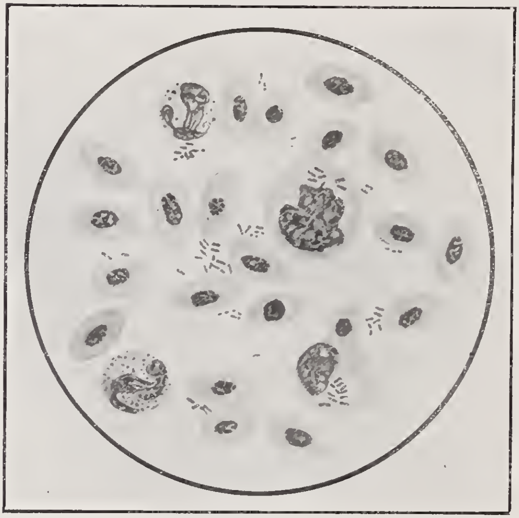


FIG. 21.
From two selected fields in blood smear showing Fowl Typhoid bacteria and leucocytosis. From drawing.

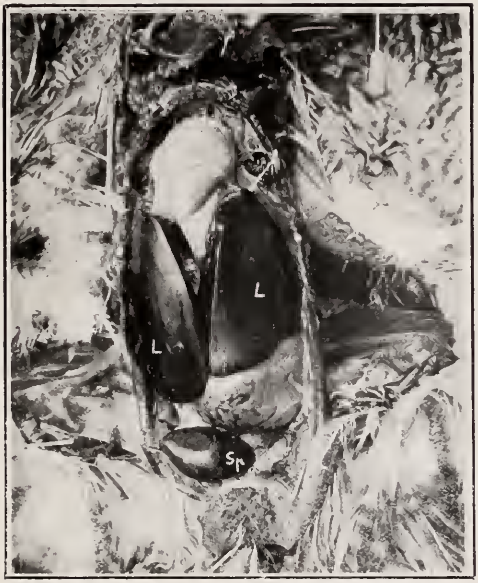


Fig. 22.
Fowl Typhoid, autopsy showing: (L) enlarged liver, and (Sp) Spleen, artificial infection.

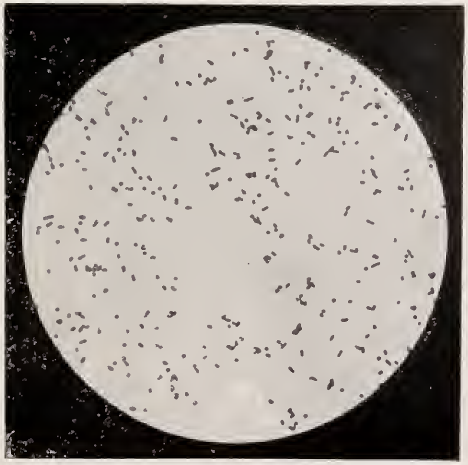


Fig. 23.
Fowl Typhoid *S. gallinarum* first generation. × 1000.

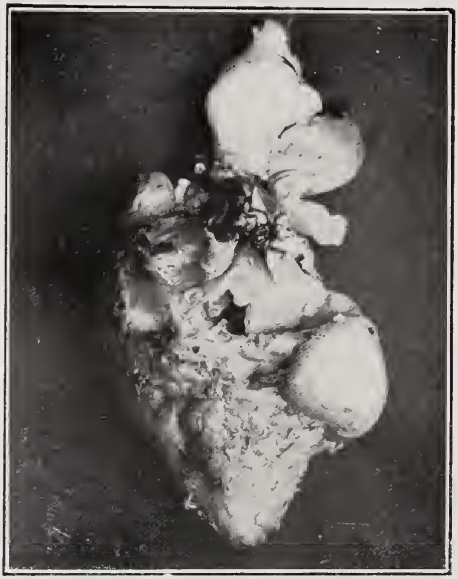


FIG. 24.

Tumour on heart, cyst on auricle and myo-pericarditis, from which *S. gallinarum* was isolated. Three months after artificial infection. See Fig. 32.

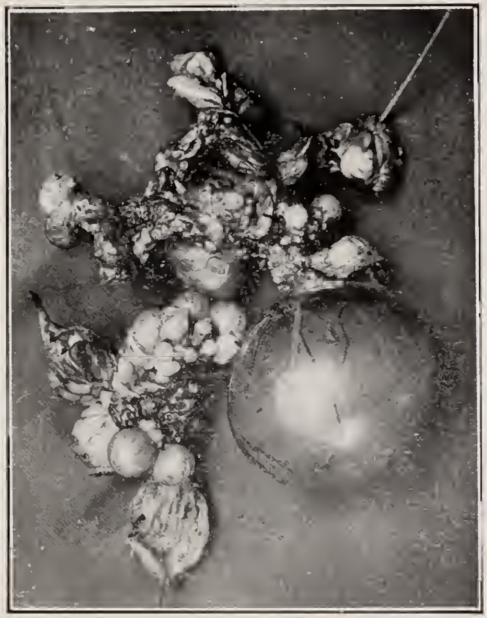


Fig. 25.
Diseased ovaries in experimental Fowl Typhoid, from which *S. gallinarum* was isolated. Three months after artificial infection.

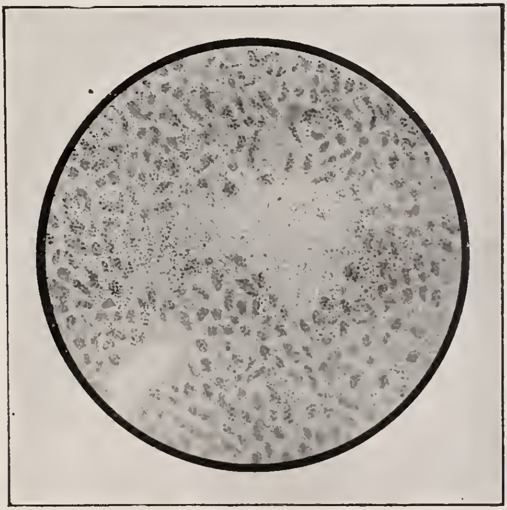


Fig. 26.
Liver. Fowl Typhoid focus, partial necrosis, showing round cells (Drawing.)

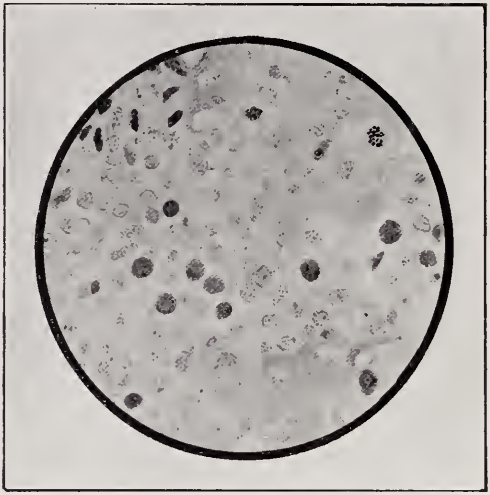


Fig. 27.

Liver. Fowl Typhoid focus, partial necrosis, showing round cells and eosinophilic leucocytes.

(Drawing.)

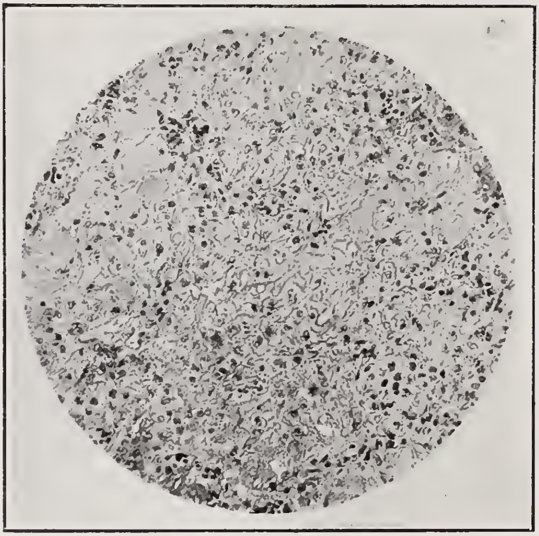


Fig. 28.

Liver. Fowl Typhoid. Portion of focus showing cellular remnants and coagulation necrosis.

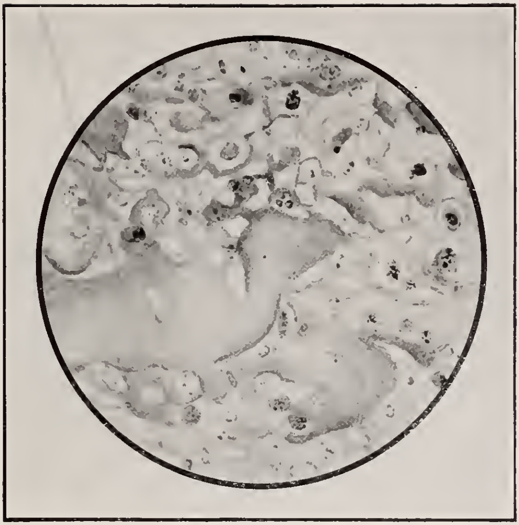


Fig. 29.
Same as above. Higher magnification.
(Drawing.)

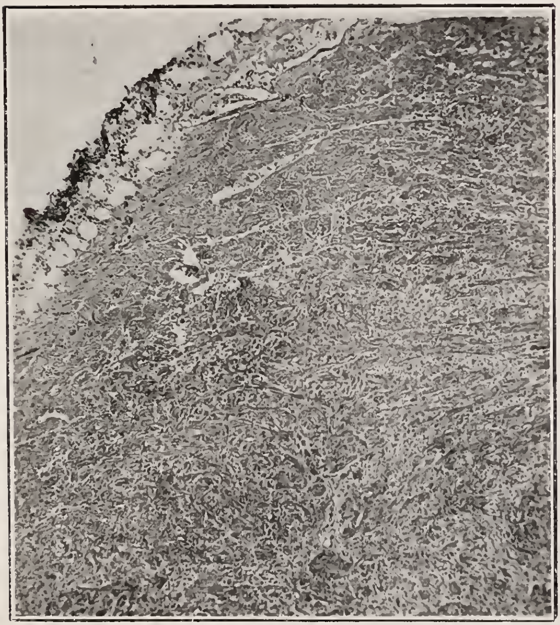


Fig. 30.
Myocardium natural infection of Fowl Typhoid showing myo-pericarditis and extensive cellular infiltration.
(Drawing.)

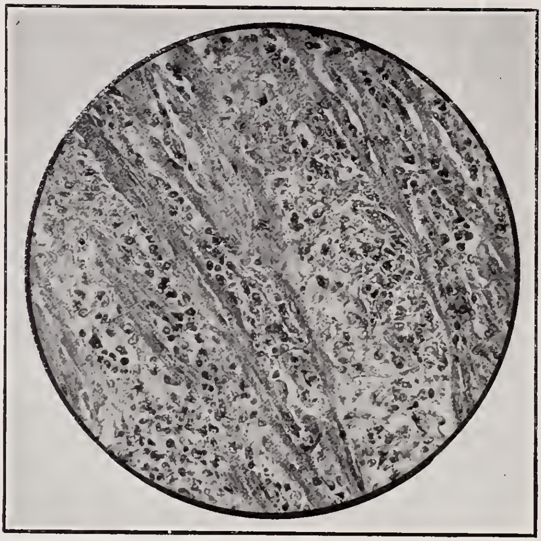


FIG. 31.
Same as above. Higher magnification. × 250.

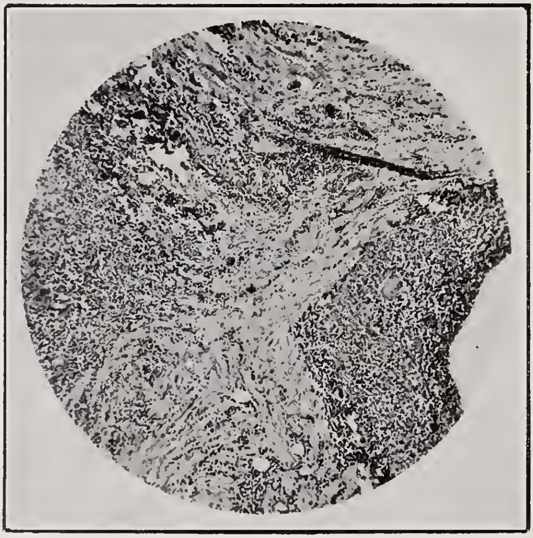


FIG. 32.
Heart lesion in experimental Fowl Typhoid three months after infection. Note cellular infiltration and atrophy of musculature.

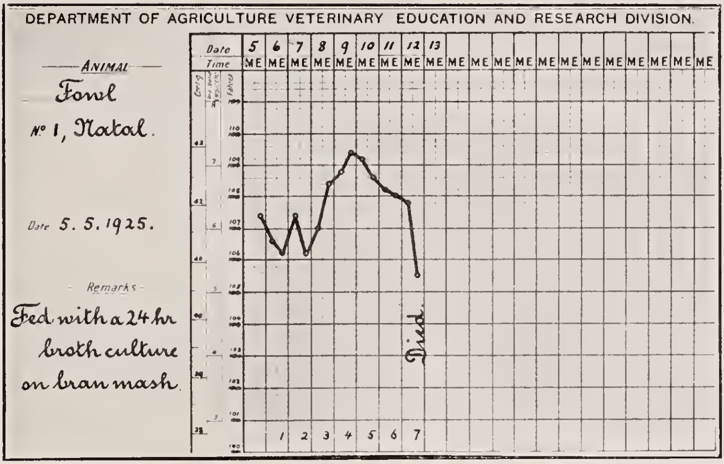


CHART 5.

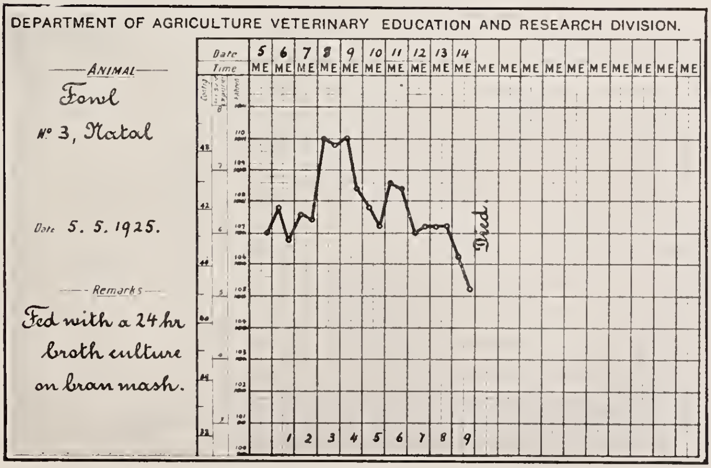


CHART 6.

Section IV.

Parasitology.

H. O. Mönnig ...	The Guinea Worm of the Ostrich, *Contortospiculum spicularia.*
H. O. Mönnig ...	*Hypodontus macropi,* n. gen., n. sp. A Hookworm of the Kangaroo.
H. O. Mönnig ...	*Filarinema flagrifer,* n. gen., n. sp. A *Trichostrongylid* Parasite of the Kangaroo.
H. O. Mönnig ...	*Agriostomum equidentatum,* n. sp. A Hookworm of the Springbuck.
H. O. Mönnig ...	Investigations into the Life-History of the Tapeworm, *Moniezia expansa.*
H. O. Mönnig ..	*Physaloptera canis,* n. sp. A New Nematode Parasite of the Dog.
F. Veglia and P. L. le Roux	Morphology of a Schistosome (*Schistosoma Mattheei,* sp. nov.) from Sheep in the Cape Province.
P. L. le Roux ...	Remarks on the Habits and the Pathogenesis of *S. mattheei*, together with the Notes on Pathological Lesions observed in Sheep.
P. L. le Roux ...	Notes on the Life-Cycle of *Schistosomum mattheei.*
P. L. le Roux ..	Two Species of *Haemonchus*, Cobb, 1898, Parasitizing the Camel in the Cape Province.
P. L. le Roux ...	A Preliminary Report on Three New Members of the Genus *Haemonchus*, Cobb, 1898, from Antelopes in South Africa.
P. L. le Roux ...	On an Oesophagostome (*Oesophagostomum susannae*, sp. nov.) from the Springhare (*Pedetes caffra*), together with Remarks on closely related Species.
P. L. le Roux ...	On a Hookworm (*Agriostomum gorgonis,* sp. nov.) from the Blue Wildebeest (*Gorgon taurinus*) in the Transvaal.
G. A. H. Bedford	Notes on some South African Ticks with Descriptions of three New Species.
G. A. H. Bedford	Anoplura (*Siphunculata* and *Mallophaga*) from South African Hosts.
G. A. H. Bedford	The Effect upon Ticks of Dipping Cattle regularly at short intervals in Arsenical Baths.

*15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.*

The "Guinea Worm" of the Ostrich, *Contortospiculum spicularia.*

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Research Officer, Onderstepoort.

THIS worm was first described by Neumann (1909)* under the name of *Filaria spicularia*, the material having been obtained from Mr. J. D. Borthwick, ex-Chief Veterinary Officer of the Union of South Africa, from South African ostriches.

The parasite has received little attention since that time and is not mentioned in most textbooks. Obviously it does not belong to the genus *Filaria* as the latter has been defined in recent works, but apart from this the original description gives quite a fair picture of the parasite.

On account of the apparent general uncertainty and hesitation to accept and to place this worm, the material available at Onderstepoort was studied, and the following account is given of it:—

Contortospiculum spicularia (Neumann, 1909).
Syn. *Filaria spicularia* Neumann, 1909.

Very large, white worms, slightly tapering towards the extremities. The cuticle has fine transverse striations and its thickness corresponds to the size of the worm.

The worms taper only slightly at the anterior end. The mouth is terminal, oval, with its longest diameter dorso-ventral, and is surrounded by a large cuticular plate from which there projects on either side of the mouth a lip-like process. In some specimens one or both of these processes may be bifid (Fig. 1). They are .032 mm. high and stand .12 mm. apart. They are flattened medially and convex laterally. The cuticular plate is irregularly oval, its long diameter measures .44 mm. in a large female, and it ends laterally on either side of the mouth in an epaulette-like, trilobed process. Each of these processes bears a papilla, the submedian papillae being larger than the lateral. There are on each side also two smaller papillae in the notches between the processes and another two small papillae near the bases of the submedian processes, making a total of seven papillae on either side (Fig. 2). The cuticular plate is surrounded by a rim of cuticle which is smooth, slightly thicker than the rest of the body cuticle and thus marked off from the latter.

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* NEUMAN, L. G., "La Filaire de l'Autruche (*Filaria spicularia*, n. sp.)," *Revue Vétérinaire*, Toulouse, 34, (9), 544-549, 1909.

Neumann describes only the four large submedian papillae and considers the two lateral papillae to be small openings connected with the lateral lines. He also says: "La périphérie de l'écusson est occupée par une zone ondulée, formée de trés fines aspérités rayonnantes," which is probably the smooth rim of cuticle through which the muscle fibres are visible.

The mouth opens immediately into the oesophagus, which has a short muscular portion, 0.68 mm. long, of conical shape, followed by a very long, thick glandular portion measuring 67 mm. by 1.2 mm., which narrows suddenly to join the intestine, a delicate tube 0.46 mm. wide, which runs straight through the body to the anus. These measurements were taken from a large female after dissection.

The male is up to 34 cm. long and 1.3 mm. thick. The hind end (Fig. 3) is slightly curved ventrad and bears medium-sized caudal alae which meet behind the tip of the tail and form a circular expansion 0.5 mm. in diameter. The cloaca has salient lips and is surrounded by four pairs of pedunculated pre-cloacal papillae and one pair of pedunculated post-cloacal papillae which support the caudal alae. Near the tip of the tail and not supporting the alae there are on either side another two small papillae, of which one stands lateral to the other and has a short peduncle. Neumann describes only one pair of papillae at the tip of the tail.

There are two spicules. The left is 1.5 mm. long, stout and somewhat twisted, it has alae which are rolled inwards so as to form a tube and bears fine tubercles in about the middle third of its length. The head of the spicule is knobbed and the point is sharp. The right spicule is 1.1 mm. long, stout, alate, and bears about 11 strong corrugations, resembling the teeth of a saw, on the ventral aspect of its distal half. The head is knobbed, the point is not quite sharp and is curved slightly ventrad. Neumann describes the left spicule as simple, homogeneous, 1 mm. long and ending in a point at either end; it is evident that he did not see the whole spicule.

The female measures up to 213 cm. long and 2.5 cm. thick. The tail is blunt and the anus, which is very minute, is situated 0.2 mm. from the extreme end. The vulva is situated 1.8 mm. from the anterior extremity; it is a large, oval, transverse opening with salient lips. Three females were dissected in order to study the genital organs. The vagina has a thick wall and runs backwards; it is 1.2 mm. long and 0.68 mm. thick. It is followed by the ovejectors, which vary much in length and in the proportions of their different parts (Fig. 4). At first there is a common portion 1.9-3.6 mm. long, which has a wide lumen and forms a small reservoir for eggs. The lumen then divides either immediately, as in Fig. 4, or a short distance further on, and forms two narrow canals which still lie in a common wall, this portion measuring 3.5-5.4 mm. long and 0.72 mm. thick. This arrangement is rather unusual for a nematode, but was found in all three females that were dissected. The two ovejectors then separate and the separate portions are 7.8 to 15.5 mm. long and 0.43 mm. thick, sometimes containing eggs which cause local dilatations of the tubes. When the separate portions of the ovejectors are short, they run straight back to join the uteri, which are wide, thin-walled tubes, also directed backwards. When the separate portions are long, one may form a loop forward, almost reaching the vagina, and then turn back again. One ovary lies anteriorly in the body, the other posteriorly. The eggs have thick shells and are short oval in shape, measuring .063-.067 mm. by .043-.049 mm. They contain fully developed embryos when laid.

The parasites are found in the peritoneum of the host, where they lie, sometimes in large numbers together, forming numerous intricate coils in the much thickened subperitoneal connective tissue. In one case eleven females and five males were removed from one area. The parasites do not seem to impair the health of the host to any great extent.

Affinities.—According to the above description, the parasite does not agree completely with the definition of the genus *Contortospiculum*, or rather with the other species referred to this genus. The two small head-papillae near the bases of the submedian processes have not been described in the other species, the large spicule is not strongly corrugated as in the other species, and the small spicule is unusually rough. The main characteristics of the genus are, however, present, viz., large size, especially of female, the characteristics of the mouth, lip-like processes, the other head papillae, oesophagus, tail of the female, the caudal alae and papillae of the male and the position of the vulva. The genital organs of the female are opisthodelph, but the occasional loop of the one ovejector may indicate an original amphidelph arrangement.

It is interesting to note that the type species of the genus *Contortospiculum rheae* (Owen, 1843) is a parasite of *Rhea americana*, a close relative of the ostrich, and that the two parasites are very much alike, although the two hosts are at present very far separated from each other geographically and must have been so since the break occurred in the southern connection of the African and American continents.

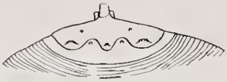

FIG. 1.

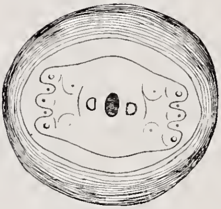

FIG. 2.

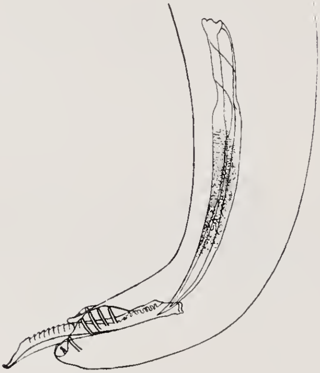

FIG. 3.

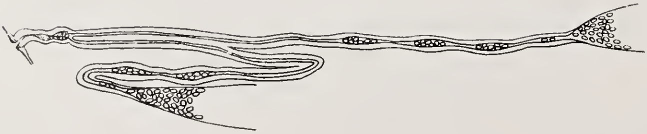

FIG. 4.

15th Annual Report of the Director of Veterinary Services, Union of South Africa. October, 1929.

Hypodontus macropi, n. gen., n. sp., A Hookworm of the Kangaroo.

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Veterinary Research Officer, Onderstepoort.

A RED KANGAROO (*Macropus rufus*) received from Australia at the Zoological Gardens, Pretoria, on the 4th December, 1928, died of a bacterial infection at the Onderstepoort Laboratory on the 2nd April, 1929. At post-mortem examination, 15 hours after death, six males and 18 females of a hookworm were found free in the intestine, some being in the posterior part of the small intestine and others in the large intestine around the ileo-caecal valve.

The worms were of a greyish-white colour, the posterior fourth of the females being light-brown. The body is not much attenuated at the extremities. The cuticle shows well-marked transverse striations.

The anterior extremity is bent towards the ventral side and the mouth opening is directed antero-ventrally. The buccal capsule is well developed and infundibular in shape. Although it opens at the side opposite to what is usual in hookworms, its general structure is much the same as in the *Necatorinae*, and the homology of the parts provides ample scope for speculation.

The mouth opening is oval and is surrounded by two lateral and four submedian papillae. The anterior, dorsal margin of the buccal capsule bears two large cutting plates, while the ventral border is notched in the middle, like the dorsal border of the capsule in other hookworms, and more laterally bears on each side a papilliform forward projection (Fig. 3). There is a short but well-developed dorsal gutter through which the dorsal oesophageal gland opens on the dorsal wall of the buccal capsule (Fig. 2), and from the anterior extremity of this gutter a Y-shaped furrow runs forwards, the arms of the Y running around the capsule in a forward direction and ending at the oral margin of the capsule at the lateral edges of the cutting plates (Figs. 2 and 3). The oesophageal funnel is large and has a strong cuticular lining, which is prolonged anteriorly into three pointed teeth projecting into the buccal capsule; one tooth corresponds to each of the three sections of the oesophagus.

The oesophagus is relatively long and slightly club-shaped. Its anterior half is surrounded by large cervical glands.

The *males* measure 13.3 to 14.8 mm. in length, with a maximal thickness of 0.46 mm. The cervical papillae are distinct; the distance from the anterior extremity to these papillae is 0.9 mm., to the excretory pore 0.83 mm., and to the nerve ring 0.65 mm. The oesophagus is 1.3 mm. long. The bursa is well developed but not very wide. The ventral rays are close together and equal; the antero-lateral is separated from the medio-lateral and is slightly larger, the medio- and postero-laterals lie close together; the stem of the lateral rays bears a large backwardly directed projection near its base. The externo-dorsal rays originate from the base of the dorsal stem; the dorsal ray bifurcates at its middle and each branch again divides into a long medial and a shorter lateral branch. Prebursal papillae are present and conspicuous.

The spicules are 1.06 mm. long and each bears a large cuticular wing; they are slightly twisted and their tips are bound together through the fusion of their alae. An oblong, shield-shaped gubernaculum is present, measuring 0.09 mm. in length.

The *females* are 17.5 to 20 mm. long with a maximal thickness of 0.57 mm. The oesophagus is 1.6 mm. long. The distance from the anterior extremity to the cervical papillae is 1.02 mm., to the excretory pore 0.96 mm., and to the nerve ring 0.77 mm. The hind end of the female begins to narrow suddenly a short distance in front of the vulva and the tail ends in a blunt point. The anus is situated 0.12 mm. from the tail end and the vulva an equal distance in front of the anus. The vagina runs forwards and is 0.23 mm. long. It opens into two forwardly directed ovejectors 0.77 mm. long, which are connected to the forwardly directed uteri. The eggs are thin-shelled and were all unsegmented; they measure 0.176 mm. × 0.078 mm.

Host.—*Macropus rufus.*

Location.—Intestine.

Locality.—Pretoria Zoological Gardens, from Australia.

Type specimens deposited in Onderstepoort Helminthological Collection, No. 2252.

Affinities.—It is obvious that this worm belongs to the *Ancylostomidae*. The genus *Strongylacantha*, which has its mouth-opening antero-ventral, is placed in the subfamily *Strongylacanthinae*, but in this worm there are large ventral teeth directed outwards, and the worm described here is obviously much more closely related to the *Necatorinae* than to *Strongylacantha*, although in the former group all the species have the mouth-opening antero-dorsal. It seems most natural to place the genus *Hypodontus* in the subfamily *Necatorinae*, which will require a slight modification of the definition of that group as follows:—

Necatorinae Lane, 1917: *Ancylostomidae* with mouth usually directed antero-dorsally. Buccal capsule infundibular, the oral margin bearing cutting plates, without teeth.

Hypodontus, n. gen., *Necatorinae*: mouth opening antero-ventrally; buccal capsule with dorsal cutting plates at oral margin and a dorsal gutter; oesophageal funnel gives rise to three teeth at base of buccal capsule. Male: bursa symmetrical, ventral rays equal and parallel, antero-lateral separated from medio- and postero-laterals, lateral stem bears a backwardly directed process, externo-dorsal arises from base of dorsal stem, dorsal ray bifurcates twice. Spicules equal, gubernaculum present. Female: vulva near anus, prodelph. Parasites of marsupials.

Type species *H. macropi* n. sp.

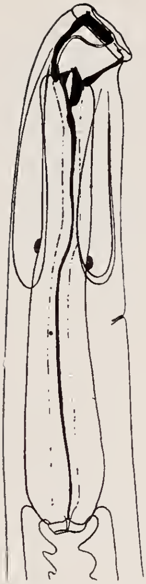

FIG. 1.
Hypodontus macropi, anterior end, lateral view.

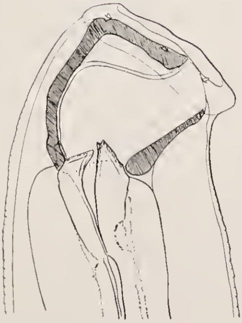

FIG. 2.
Hypodontus macropi, head, lateral view.

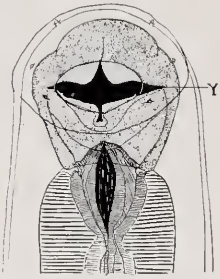

FIG. 3.
Hypodontus macropi, head, ventral view. Y=termination of Y-shaped groove.

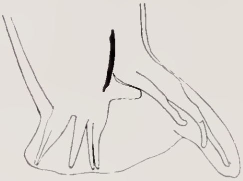

FIG. 4.
Hypodontus macropi, male bursa, lateral view, showing gubernaculum.

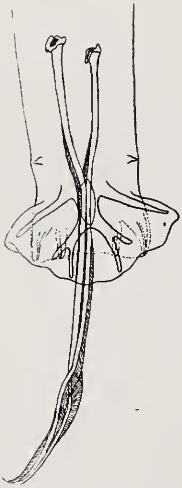

FIG. 5.
Hypodontus macropi, male bursa, dorsal view.

*15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.*

Filarinema flagrifer n. gen., n. sp., A Trichostrongylid Parasite of the Kangaroo.

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Veterinary Research Officer, Onderstepoort.

On the 6th June, 1929, a red kangaroo received from Australia on the 4th December, 1928, died in the Zoological Gardens at Pretoria. The animal harboured a large number of *Rugopharynx australis* in the stomach, together with 16 females and 5 males of a worm that appeared to be unknown.

The worms had a light reddish-yellow colour when fresh and are probably bloodsuckers. They are thread-like in appearance, tapering more towards the anterior extremity than towards the posterior, and are not unlike *Haemonchus contortus* in shape.

The cuticle shows extremely fine transverse striations and no longitudinal striation. In most of the specimens it was irregularly dilated, but since this was not the case in a few worms of both sexes, the dilatation is taken to be the result of decomposition, since the carcase was not fresh.

The head is not marked off from the body and is very narrow, in the male .021 mm. wide. The mouth is surrounded by two lateral and four smaller submedian papillae. The oesophagus begins immediately behind the mouth-opening and contains anteriorly a dilatation of its lumen surrounded by a cylinder of thick chitin, which appears like a bar of chitin .02 mm. long, on either side of the dilatation, when seen in one plane (Fig. 1). The oesophagus becomes slightly thicker backwards and has its opening into the intestine guarded by the usual three valves. The intestine is narrow and not pigmented.

The *males* measure 15.8 mm. to 17.8 mm. in length and are about 0.26 mm. thick. The oesophagus is 1.8 mm. long and is surrounded by the nerve ring 0.58 mm. from the anterior end of the body. The cervical papillae are very small and flat, situated 0.65 mm. from the anterior end. The excretory pore is distinct and 0.6 mm. distant from the head end.

The bursa of the male is well developed; especially the anterior, ventral portions of the lateral lobes are large and prominent, giving the bursa a rectangular appearance in a lateral view (Fig. 2). The outer surface of the bursa is covered with fine markings on the cuticle, which are not unlike finger-prints. Prebursal papillae are present, but small and flat. The ventro-ventral ray is a little smaller than the latero-ventral and turns forwards, almost at a right angle, to support the large anterior portion of the bursa; the latero-ventral ray runs more or less parallel to the lateral rays. The latter are about equal in size and slightly divergent from one another. The externo-dorsal rays arise from the base of the dorsal stem and their proximal portions are swollen, but they soon narrow down and their tips do not quite reach the edge of the bursa. The dorsal ray proper bifurcates at about the middle of its length and the tips of the branches are bidigitate.

There are two equal and similar spicules (Fig. 4); each ends in a narrow point, while from about the middle of each spicule there arise, not quite at the same height, two whip-like processes which end at the same level and some distance beyond the main point of the spicule. The spicules have small alae, which are visible especially in the proximal half and near the tips. The length of the spicule proper is about .44 mm., and the whip-like processes extend about 0.58 mm. further. The gubernaculum is large, a long oval in shape, and slightly concave ventrally; it is about .25 mm. long.

The genital cone is short. Ventrally to the cloacal aperture there is a small, blunt median process bearing on either side a small pointed projection. Dorsally there is on either side a larger triangular process.

The *females* measure 31 mm. to 33.5 mm. in length with a maximum thickness of .33 mm. The nerve ring, excretory pore, and cervical papillae are situated at about the same distances from the anterior end of the body as in the male. The oesophagus is 2.03 mm. long. The hind end of the female narrows down gradually and ends in a blunt tail, which bears a pair of small lateral papillae .06 mm. from the tip. The anus is situated .43 mm. from the tip of the tail.

The vulva is not prominent and is placed about 11.25 mm. in front of the hind end, at the beginning of the posterior third of the body. The vagina is transverse and short, the ovejectors proceed in opposite directions, they are short and have the usual structure and the uteri are opposite. The eggs have thin shells and are segmenting when laid; they measure on the average .153 mm. × .074 mm.

Host.—Red kangaroo (*Macropus rufus*).

Location.—Stomach.

Locality.—Pretoria Zoological Gardens, recently from Australia.

Type species in Onderstepoort Helminthological Collection No. 2253.

Affinities.—Obviously this worm belongs to the Trichostrongylinae. It does, however, not belong to any one of the known genera of that family according to the way these are defined and a new genus has to be created for it.

GENERIC DIAGNOSIS.—*Filarinema—Trichostrongylinae*: slender worms with narrow head; cuticle not inflated around the head and with fine transverse striations, without longitudinal striation; cervical papillae and prebursal papillae of male small. Male: bursa with large lateral and small dorsal lobes; ventral rays unequal and divergent; lateral rays approximately equal and slightly divergent from one another; externo-dorsal arises from base of dorsal, dorsal bifurcates at its middle, each branch bidigitate. Spicules, equal, similar, bearing each two whip-like processes; gubernaculum present and large. Female: vulva in posterior half of body; amphidelph; oviparous. Parasites of Marsupials.

Type species: *F. flagrifer*, n. sp.

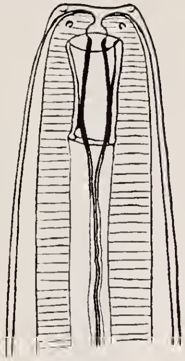

FIG. 1.
Filarinema flagrifer—head end.

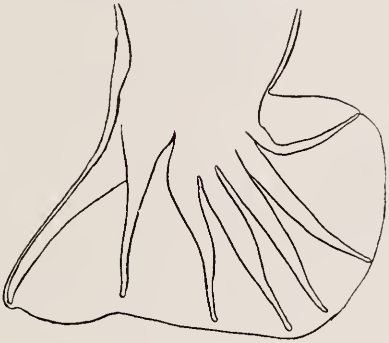

FIG. 2.
Filarinema flagrifer—male bursa, lateral view.

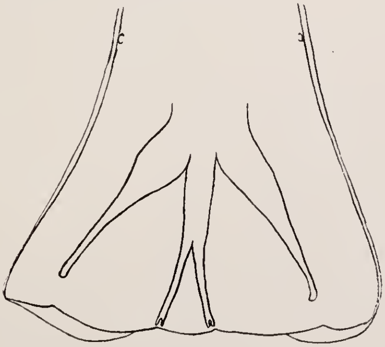

FIG. 3.
Filarinema flagrifer—male bursa, dorsal ray.

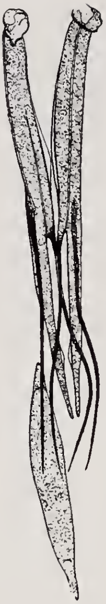

FIG. 4.
Filarinema flagrifer, spicules and gubernaculum.

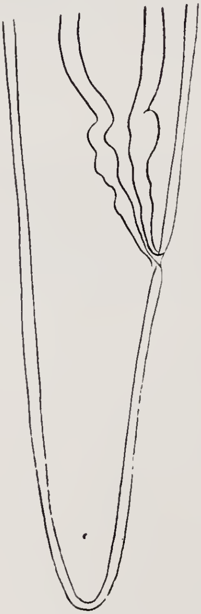

FIG. 5.
Filarinema flagrifer, tail of female.

*15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.*

Agriostomum equidentatum, n.sp., A Hookworm of the Springbuck.

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Veterinary Research Officer, Onderstepoort.

THE worms described here were taken from the small intestine of a Springbuck (*Antidorcas marsupialis*) near Aliwal North in the Cape Province in June, 1929. They were firmly fixed to the mucous membrane. The worms have a reddish-grey colour and a darkly pigmented intestine. The body tapers towards the posterior extremity, but not much anteriorly. The cuticle is marked with fine transverse striations. A ventral cervical groove, as described in *A. vryburgi*, is not present.

The head end is bent dorsad and the mouth-opening is situated antero-dorsally. It is surrounded by two lateral and four submedian papillae. The buccal capsule is large and heavily chitinized and its size is increased by the presence of a large oesophageal funnel, which forms a continuous cavity with the buccal capsule, the two together giving the appearance of a true buccal capsule (Fig. 1). The entrance into the cavity is armed with seven double teeth arranged as follows: one ventral, a pair ventro-lateral, a pair lateral, and a pair dorso-lateral (Fig. 2). The two points of each tooth are equal in size, and in this respect this species differs from *A. gorgonis* Le Roux, in which the median points are smaller than the lateral. The only other species of the genus, *A. vryburgi* Railliet, has apparently single teeth, although their arrangement is not quite clear from the available descriptions and should be studied again. Dorsally, as is usually in hookworms, the buccal capsule bears a small prominence, which is cleft in the middle. Near the anterior margin there is, on the inner surface of the buccal capsule, a circular furrow as described by Looss for *Ancylostoma duodenale*. A similar structure is described in *A. gorgonis* and in *A. vryburgi*.

The dorsal wall of the oesophageal funnel bears a ridge, which is a dorsal gutter, and the apex of which projects into the lumen of the cavity in the form of a dorsal tooth. Along the ventro-lateral walls of the oesophageal funnel there runs on each side a ridge, the two forming a V-shaped structure as seen in a ventral view, beginning from the base of the funnel. Each ridge rises up to form a ventro-lateral tooth in the oesophageal funnel. These structures in the funnel are not present in *A. gorgonis* and apparently also not in *A. vryburgi*.

The junction between the buccal capsule proper and the oesophageal funnel is indicated by a ridge encircling the cavity; this is a thickening of the cuticular wall on the outside, which has irregular edges and serves for the attachment of the oesophageal muscles.

The oesophagus behind the funnel is club-shaped and strongly muscular, and its entrance into the intestine is guarded by the usual three valves. The intestine is darkly pigmented, wide, and irregular in outline.

The *males* vary very much in length, from 5 to 10 mm.; a similar variation was found in the females, and, since the latter contained no eggs, the specimens may not be quite mature. The greatest breadth of the males is .44 mm. The buccal capsule and oesophageal funnel together are .4 mm. long and .31 mm. wide in the largest male, from which also all the following measurements were taken. From the anterior extremity to the cervical papillae the distance is .95 mm., to the excretory pore .81 mm., and to the nerve ring .69 mm. The cervical papiliae are very fine and inconspicuous. The portion of the oesophagus posterior to the funnel is 1 mm. long. The bursa (Figs. 3 and 4) is not very large, and the lateral lobes are curved in towards the ventral median line. The dorsal lobe is small. The ventral rays are equal and parallel, reaching the margin of the bursa, and are widely separated from the lateral rays. The antero-lateral ray is short and stout, slightly separated from the other two laterals, which lie close together, and it curves outwards so as to lift a small part of the bursa into a projecting point about half-way down to the margin. The medio- and postero-lateral rays reach the edge of the bursa. The externo-dorsal rays arise at the end of the first third of the dorsal stem; they do not reach the edge of the bursa and their tips turn up to the dorsal surface. The dorsal ray bifurcates and each branch divides into a longer median and a shorter lateral branch. The prebursal papillae are prominent and pierce through the cuticle.

There are two equal alate spicules, 1.52 mm. long. They end in fine points surrounded by a hyaline membrane and the alae end near the tip in an abrupt way so as to leave a free alar point; this can only be properly seen when the points of the spicules protrude from the cloaca or when the spicular apparatus is dissected out.

The spicules are guided by a large telamon, .27 mm. long, which is very transparent and the structure of which is difficult to make out (Fig. 5, 6, and 7). Staining and dissection were resorted to in order to study the telamon in this species. Proximally there is a fairly thick hood-shaped body which lies between the spicules; the body has a dorsal expansion which curves outwards over the spicules on either side; ventrally the body gives off two long posteriorly directed arms which lie ventro-laterally to the spicules and each arm has a wing-like expansion which curves up around the spicule towards the dorsal side. A gubernaculum is not present; in a lateral view there appears to be a small gubernaculum, but on rolling the worm this disappears and the structures responsible for this illusion are two thickenings in the distal wall of the spicule sheath and the wings of the arms of the telamon, which are in certain positions seen in a line. A telamon as described above was found also on re-examination of the material of *A. gorgonis* and no gubernaculum is present in this species. It seems very necessary that, also with regard to this point, the third species, *A. vryburgi*, should be re-examined.

The *females* measure 7.3 to 13.3 mm. in length; greatest breadth .55 mm. The distance from the anterior end to the cervical papillae is .95 mm., to the excretory pore .79 mm., and to the nerve ring .75 mm. The buccal capsule and oesophageal funnel are .42 mm. long and .33 mm. wide. The oesophagus posteriorly to the funnel is 1.04 mm. long. The tail is acute (Fig. 8) and the body narrows down suddenly behind the vulva. The anus is placed .33 mm. in front of the tip of the tail and the vulva .79 mm. The vagina runs forwards and is .48 mm. long. The ovejectors are short, one is directed forwards and one backwards, but turns forwards where it joins the uterus, both uteri running forwards.

None of the females contained eggs.

Host.—Springbuck (*Antidorcas marsupialis*).

Location.—Small intestine.

Locality.—Aliwal North, Cape Province.

Types in Onderstepoort Helminthological Collection, No. 2254.

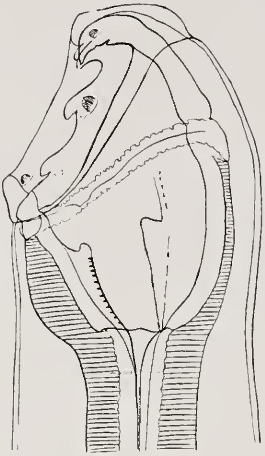

Fig. 1.
Agriostomum equidentatum—head, lateral view.

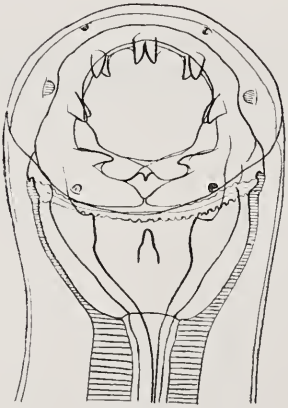

Fig. 2.
A. equidentatum—head, dorsal view.

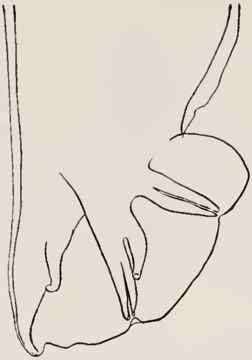

FIG. 3.
A. equidentatum—male bursa, lateral view.

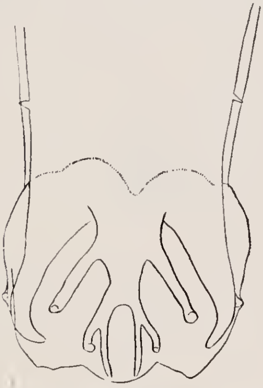

FIG. 4.
A. equidentatum—male bursa, dorsal view.

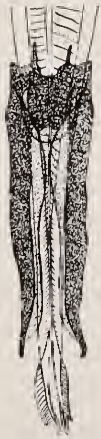

FIG. 5.
A. equidentatum—spicules and telamon, dorsal view.

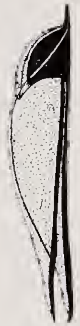

FIG. 6.
A. equidentatum—diagrammatic lateral view of telamon.

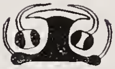

FIG. 7.
A. equidentatum—diagrammatic transverse section through body of telamon and spicules to show relative positions.

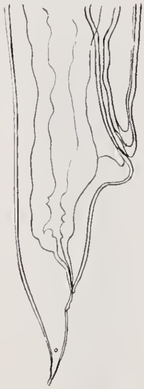

FIG. 8.
A. equidentatum—tail of female.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

Investigations into the Life-History of the Tapeworm *Moniezia expansa.*

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Research Officer,
Onderstepoort.

THE life-history of the sheep tapeworm, *Moniezia expansa*, and of other tapeworms of the family Anoplocephalidae has drawn a considerable number of parasitologists in different countries to attempt the solution of this elusive problem. The results so far have been entirely on the negative side, although such negative results are, of course, quite valuable to guide further research. The following is a resumé of some more negative results on this subject, published in the hope that it may aid other workers in this field, at least in so far as to point out to them a few more fruitless lines.

The theoretical possibilities of the question how lambs become infected may be stated as follows. Infection may occur:

I. Prenatal.
II. Postnatal.
  A. Without intermediate host.
  B. With intermediate host.
    a. From the ewe, through the milk.
    b. From the outside of the udder.
    c. From the skin of the lamb.
    d. From the grass, soil, or water.
    e. Through blood-sucking invertebrates.
    f. Through the air.

In cases *b*, *c*, *d*, and *f* the intermediate stage would be taken in with the intermediate host (primary or secondary) or with the faeces of the intermediate host, the eggs of the worm having become infective on passage through the alimentary canal of such an intermediate host.

A fairly comprehensive account of the literature on the subject is given by Flattely (1922), and, although it is, therefore, not intended to repeat such a resumé here, it may be helpful to consider the above-mentioned possibilities in the light of the knowledge which has been gained on this subject in the past.

I. *Prenatal infection* must be considered since it is well known that lambs can pass ripe segments of the tapeworm at a remarkably early age. If infection is not prenatal, the growth of the worm must be extremely rapid in comparison with other tapeworms of about the same size, like *Taenia solium*, which takes 11 to 12 weeks to grow adult, while lambs of six weeks old may pass ripe segments of *Moniezia expansa.*

Our experience, however, tells strongly against prenatal infection. In the first place, prenatal infections by Cestodes are very rare, much more so than in the case of Nematodes. This can probably be explained by the fact that it is much more difficult for a hexacanth Cestode embryo to pass the placental barrier than for the much more active Nematode larvae. Extremely heavy *Moniezia* infections are sometimes met with, and it is difficult to imagine that they come about by prenatal infection. Secondly, very young tapeworms have been found in lambs several months old and even in full-grown sheep. Morgan (1924) mentions a case in which he found two specimens measuring 5 mm. and 8.5 mm. in a three-year-old wether. The writer found in a three-months-old lamb 52 specimens of *M. expansa* measuring from 1 cm. up to 2 metres long. This tends to prove that the lamb had been subject to a constant infection, and that infection was not prenatal unless certain unknown conditions in the intestine of sheep may sometimes retard the rate of growth of the tapeworm, but which has not been proved to take place.

II. *Postnatal infection.*—A. *Without intermediate host.*—Several workers, especially Megnin (1883) and Curtice (1890), have maintained that infection does not occur through the agency of an intermediate host. When one is investigating this problem, and frequently ponders over the possibilities and probabilities of it, one is sometimes inclined to attach some weight to this argument. Investigations have been carried out by several workers on heavily infected farms; the invertebrate fauna has been studied and numerous species have been fed with *Moniezia* eggs, or collected on infected areas and dissected, but, although lambs are constantly becoming infected, there has never been found the slightest indication of an intermediate stage in any invertebrate. The family Anoplocephalidae comprises quite a number of different species, parasitic in a variety of animals, and many workers have dissected numerous pecies of invertebrates in the quest for intermediate stages of helminths, but have never seen an intermediate stage of an anoplocephalid, unless the cysticercoid recently described by Joyeux and Kobôzieff (1928) from the beetle *Geotrupes sylvaticus* should be one.

Even though the anoplocephalid intermediate stage should, contrary to expectation, not look at all like a cysticercoid, it would, in any case, comprise the head of the tapeworm and must be at least as large as that, which is not very small, so that one ought to find it in a careful dissection of an invertebrate where one searches for it.

After such considerations, one feels inclined to argue that, if the lambs can find the intermediate stage so readily, why not the investigator? And the conclusion is very tempting: that there is no intermediate host—a state of affairs wholly unknown amongst Cestoda. The eggs may, of course, first develop to an intermediate stage in the lamb itself, as happens in certain species of *Hymenolepis.* However, it has never been possible to infect lambs by feeding *Moniezia* eggs to them, even after such eggs had been given every likely opportunity to mature, if necessary. Some of the authors who have attempted direct infection are Curtice (1890), Stiles (1893), and Flattely (1922). The writer also fed some eggs fresh and others after they had been kept on moist sand and moist blotting-paper in the laboratory for periods up to six weeks, but no infection resulted. The eggs did not seem to alter at all during the time they were kept in this way.

B. *With intermediate host.*—After the above arguments it seems still most probable that there must be an intermediate host, but the type of life-history is perhaps different from the ordinary type of life-history found in other cestodes, or, in other words, there is perhaps something unusual in the development of these tapeworms which we do not at all expect and which has been the cause of all our failures so far. As has already been mentioned above, and also previously by other writers—Lebour (1915), Flattely (1922)—the *Moniezia* eggs may become infective by passing through the alimentary canal of some other animal and would then have to be swallowed by the lamb with the faeces of such an animal, or with dust, grass, water, etc.

a. From the ewe, through the milk.—It is popularly believed that lambs become infected through the milk of the ewe, and in South Africa *M. expansa* is frequently called "Milk-worm." The idea here is that an intermediate stage may develop in the udder tissues of the ewe and pass into the milk. Flattely did a considerable amount of work to test this possibility, but was not able to find anything, neither in the milk nor in the tissues of the udders of ewes. The following facts may also be considered: Lambs reared on cows' milk from the third day, and in some cases even from birth, have been known to the writer to become just as heavily infected as lambs reared by their mothers, while the calves of the cows supplying the milk were not, or only very slightly, infected. It may be that the calves were not as susceptible as the lambs, but heavy infections with *M. expansa* in calves occur frequently. The calves in these cases did not run on the same pastures as the lambs. Further, if infection occurs through the milk, one would not expect a temporary drought to sweep away the infection as completely as has been experienced by the writer. The circumstances of this experience will be related further on. It, therefore, seems likely that infection does not occur through the milk, but this possibility should still receive some further attention.

b. From the outside of the udder the lamb may ingest, while sucking, small invertebrates or infective eggs of the tapeworm. Flattely examined washings of udders on infected farms, but with negative results.

c. From the skin of the lamb.—It is frequently seen that lambs bite themselves, especially when troubled by keds. In this case the lamb may swallow an intermediate host or infective eggs. Here we think especially of the ectoparasites of sheep as possible intermediate hosts. The keds have already received much attention, especially from McMurrich (1884) and Stiles (1893), but results were negative. The writer also tried to infect keds, as will be described later, but was not able to do so. In this connection one must, however, bear in mind the fact that calves sometimes suffer from very heavy infections of *M. expansa*, and they would probably not get them through keds, which are not parasites of cattle. The same argument must be considered in connection with other ectoparasites.

d. From the grass, soil, or water.—This still seems to be the most probable source of infection, through the agency of an intermediate host directly, or indirectly through its faeces. It will be interesting here to consider some observations made by the writer, during two months devoted to an investigation of this problem, on the habits of lambs that have a bearing on the above-stated possibility. A close watch was kept on the habits and doings of young lambs and careful post-mortem examinations were made on lambs of various ages killed for the purpose. It was observed that lambs begin to lick the soil and, more rarely, the grass when they are only a few hours old. This licking habit increases during the first fortnight, and lambs killed during this period invariably had in their stomachs a considerable amount of sand, small seeds, dry grass, and bits of other dry plant material, and, in some cases, small insects. The lambs were most persistent in practising this habit and, even when muzzled, still tried their utmost to get hold of some sand. Since these lambs drank on normal ewes, one could hardly look for a deficiency behind this habit. When about two weeks old, the lambs began to nibble at the green grass, and swallowed a small amount. At the age of three weeks they can be said to eat grass properly already, the rumen frequently containing a large handful, or more, of green grass. The licking habit, however, still continues up to about five to six weeks and then decreases.

Since the infection must frequently occur soon after birth, it seems very probable that the licking habit has something to do with it, and that the infection is obtained in this way from the soil or the plants that are being licked.

On the other hand, it was never observed that lambs drank any water before they were about two weeks old, even though the weather at the time was dry and hot. When about two weeks old, the lambs begin to smell the water and to taste it and gradually begin to drink, but they never drink much before they are weaned.

There are practically no observations which would indicate whether drinking-water plays a rôle in the life-history of *M. expansa.* Curtice describes the occurrence of infection in a lamb that was given drinking-water from a well, which seems to imply that the water was considered to be clean, and therefore did not cause the infection. During the writer's experiments mentioned above, the sheep drank water from an open pond which was none too clean .A few heavily infected lambs were running with these sheep, so that there was every opportunity for the water to become infected, besides being on a farm which had been severely infected less than three months before. Of 20 lambs, however, only one acquired a tapeworm. A relatively severe drought for that time of the year was being experienced during this period, and the conclusion is very likely that this was the cause of the great scarcity of infection, and that drinking-water does not play an important part in the life-history of the tapeworm. The drought at this time may indeed be looked upon as having provided an opportunity for testing the rôle of drinking-water by excluding some other possibilities.

e. and *f. Through blood-sucking invertebrates and through the air.*—These methods of transmission, although entirely unknown amongst the Cestoda, must be considered as theoretical possibilities, however small the probabilities seem to be, and though it may not be necessary to devote any further attention to them at present.

EXPERIMENTAL WORK.

I. EXPERIMENTS AT ERMELO (TRANSVAAL HIGHVELD), FEBRUARY TO JULY, 1927.

The farm on which these experiments were carried out was at the time only moderately infected. The rainy season coincides with the summer, from October to March more or less, but that special season had been a moderately dry one. It is generally stated in that area that spring (September) lambs suffer much more frequently from tapeworm than autumn (April) lambs. Since the season was, according to these statements, not very favourable, the work done consisted chiefly of dissecting various invertebrates which had either been kept in contact with *Moniezia* eggs or collected in a camp in which infected lambs were running. A number of lambing ewes was also run in this camp with the infected lambs, but only a small proportion of the lambs born in this camp became infected. Through this camp there passes a small stream of water from which the sheep drank. At this time, however, the stream consisted of stagnant pools only.

Experiments with Keds.—Several attempts were made to feed keds with *Moniezia* eggs. A patch on the skin of a sheep was clipped short and liberally smeared over with the eggs. Keds were then placed on this patch and kept there by covering them with a cardboard ring closed on the outside with fine gauze and tied to the sheep by means of tapes. Some keds were occasionally removed and kept for a time in a clean vessel, in order to collect their faeces. The latter never contained tapeworm eggs. Keds were dissected after various periods of feeding on the infected patch, but the result was always negative.

Coprophagous Mites.—Flattely (1922) states that he intends directing attention to coprophagous mites, but he has not published anything on this subject since, as far as the writer is aware. Two species of mites were found at Ermelo in the manure of stables and sheds, and they were remarkably more frequent in the cow-sheds than in the sheep-sheds. Manure containing these mites was kept in the laboratory in large glass dishes, about 5 cm. deep, and ripe segments of *Moniezia* were added liberally. Usually after about eight days the numbers of the mites decreased rapidly and after 14 days there were very few left, even though the manure had been kept slightly moist. Mites were picked out of the manure after various intervals, and examined whole and after they had been torn to pieces by means of fine needles. Altogether 308 mites were so examined, with negative result.

Mites live by sucking fluids and their mouth parts do not allow large particles to pass; it is, therefore, rather unlikely that they could swallow *Moniezia* eggs. Nevertheless, one cannot help regarding them with some suspicion in connection with the problem before us.

Musca spp.—A large number of flies were caught and placed in a cage containing a dish of sugar-water and a dish of ripe *Moniezia* segments. The flies frequently settled on the tapeworm material and were seen to suck at it. Numerous dissections of such flies were made, but nothing was found, not even eggs in their alimentary canals. Mites found on the flies were also examined and showed nothing.

A quantity of fresh horse manure, containing no tapeworm eggs, was placed in a dish in the cage after a good quantity of *Moniezia* eggs had been added. After two days some more eggs were added and the dish was transferred to an incubator running at 26° C. Five days later a number of maggots were seen in the dish and more *Moniezia* eggs were added. In due course the maggots pupated and the flies emerged from these. Several pupae and all the flies were dissected, 36 altogether, but only a number of *Habronema* larvae were found in them. This experiment was repeated a second time, yielding 34 pupae and flies, with the same result.

Carrion Flies and Blue-bottles.—A large number of these flies, belonging to several different species, were treated in the same way as the house-flies and some were also bred from diarrhoeic sheep's faeces into which the maggots had been laid and to which *Moniezia* eggs had been added. Others were captured in the infected camp. All of them were negative on dissection and none contained tapeworm eggs in the alimentary canal, although they had sucked at the segments more often than the house-flies.

Other Invertebrates were collected in the infected camp and dissected. They were not specifically identified, but belonged to the following groups: Ants (77), dung-beetles (11), other beetles from soil and water (32), Diptera other than the flies already mentioned and dipteran larvae from water (22), a small number of grasshoppers (Orthoptera), spiders, and earwigs. These were all negative. Ripe *Moniezia* segments had been spread around the openings of the ants' nests for several days before the ants were collected. These ants were very frequent in the camp, but they were not seen to touch the tapeworm material. No small Crustacea nor any snails could be found in or near the water at that time.

In addition to these experiments with invertebrates, the lambs born in this camp were carefully examined post-mortem when they died, and some were specially killed for the purpose. The contents and mucous membrane of the stomachs were closely examined and the contents and mucous membrane of the small intestine were collected by scraping and searched through in small quantities at a time in the hope of finding the earliest stages of the tapeworm in the sheep. Only a few of the lambs were infected, and nothing smaller than 1 cm. long tapeworms were found.

II. Experiments at Amersfoort (Transvaal Highveld), September to October, 1928.

The farm on which these experiments were conducted is well known to be usually very heavily infected with *M. expansa,* and the owner considers it quite unusual for a lamb to reach the age of six weeks without infection. During a visit of the writer, three months before the experiments were started, the lambs were badly infected, owing to the failure of a certain supply of male-fern to remove the worms. It was advised to treat the lambs with the following mixture, which had previously been found to yield successful results:—

Copper sulphate, 1 oz.
40 per cent. nicotine tobacco dip, 1 oz.
Water, 3 pints.

Doses: lambs one to three months, ½ oz.; three to six months, ¾ oz.; over six months, 1 oz. This treatment was introduced immediately and very favourable results were reported. However, the writer does not think that this is the reason why the infection practically disappeared, but rather incriminates the subsequent drought.

It might be mentioned that this is a stud-farm carrying very valuable stock, which is well cared for. The size of the farm is about 8,000 acres. There are a number of fairly high hills, but only one really moist valley between the hills, but the infection occurs apparently all over and not only in this valley. On the neighbouring farms, of which one is also a stud-farm, *M. expansa* occurs in very moderate numbers only. The reason for this local severe infection is quite obscure.

It was considered a unique opportunity to attack the problem on this farm, as the owner was also greatly interested and offered his assistance liberally. The plan of the experiment was as follows: Sixty ewes in lamb were sent to the farm three to four weeks before they would start lambing and were run in a camp in which infection was known to occur, together with some infected lambs. By means of muzzling some lambs immediately after birth and allowing one-half of these to drink on washed udders only, and the other half on unwashed udders, it was intended to exclude all infection except that which could occur before birth, through the milk, from the air, from blood-sucking invertebrates, and, in one half of the lambs, from the outside of the udder. Further, it was intended to examine udder washings, milk, soil, plants of the pasture, and water, and to study the invertebrate fauna and dissect specimens after they had been kept in contact with *Moniezia* eggs. As it is important to know the earliest age at which lambs can become infected, lambs were to be killed and thoroughly examined, also in the hope of finding the earliest stages of the parasite in the lamb. Observations were to be made on the habits of young lambs and on all other factors that could have any bearing on the problem.

The Importance of Moisture.

Although it has sometimes been thought that moist conditions favour the infection, no definite observations have been recorded on this point. The experiments here described, although very disappointing on most points, bring out this fact well, that moist conditions are necessary and that a drought is very effective in checking the infection. Although the lambs of the farm had been successfully treated, a few had again become infected, and other infected lambs were introduced on to the farm for the purposes of the experiment. Yet, in spite of no further treatment, the infection did not spread as usual on that farm.

In order to understand the conditions properly, it must be explained that during the previous three years the rainfall had been relatively low and the winter preceding the experiment had been a very dry one. During the two months of the experiment, altogether 2.82 inches of rain fell as follows:—

15th and 16th September ...	0.76 inch (first rain after winter).
23rd September	0.25 inch.
30th Sept. to 1st October ...	0.75 inch.
15th to 16th October	0.92 inch.
22nd October	0.14 inch.

This does not look quite like a drought, at least for that part of the country, and it must, therefore, be added that the heavy showers which fell during a short time in most cases, and, therefore, did not soak in properly, were followed by dry winds, which very soon dried the upper layers of the soil, scorched the young sprouting grass, and left conditions much as before the rain.

The usual lambing season on this farm is about March to April, but about 120 lambs are also reared each September, and all suffer equally from tapeworms. This year the September lambs showed no tapeworm symptoms, and some treated ones passed no worms. Several faecal examinations also gave negative results. Of the lambs born of the experimental ewes, 20 unmuzzled ones were killed at various ages and only one had become infected. The infection was, therefore, very rare at this time, and this state of affairs can only be ascribed to the drought.

Muzzling Experiment.—Eight lambs were muzzled immediately after birth. The ewes were caught during parturition and the head of the lamb was not allowed to touch the ground before the muzzle had been attached. The lambs were allowed to drink at fixed times, the ewe being placed on its haunches and, after the muzzle had been removed, the lamb was held so that it should suck only at the teats. Four of the lambs were allowed to drink only after the udders had been washed, the wool around the udders having been clipped at the first drink. Eight unmuzzled lambs were marked as controls for this experiment. At the close of the experiment, when these lambs were killed, they were all between 34 and 45 days old, except three controls that had been killed at earlier stages in order to see whether infection was taking place. Only one of the controls, 44 days old, harboured one *M. expansa*, 120 cm. long, no ripe segments having yet been cast off from it. This single case of infection, being amongst the controls, signifies nothing, and the muzzling experiment may be said to have fallen quite flat. An experiment of this nature should again be carried out, and the writer hopes to repeat it in the near future. The muzzling must be done very carefully, and the air-holes in the muzzles must be very small, as it was found that the lambs were so persistent in trying to lick the soil, that they continually rubbed the muzzles along the ground, trying to get some material in through the air-holes in order to swallow it. The muzzles were made of strong canvas and leather straps, covering the whole head except the eyes, the ears, and the poll; at the sides, near the front, there were four air-holes, 1 cm. in diameter, made of strong eyelets. But all the muzzled lambs had a small quantity of sand in the stomach at post-mortem examination, showing that the air-holes had been too large.

Post-mortems on Lambs.—Besides the 16 lambs of the muzzling experiment, another 12 lambs were killed for examination, varying in age from seven to forty-one days. The lambs were usually killed at 2 p.m. after having been let out to graze at 6 a.m. Immediately after killing, the stomachs and small intestine were removed. The forestomachs, abomasum, and intestine were opened separately. The contents of the forestomachs were washed into a dish and the mucous membrane then carefully examined. The abomasum was next treated in the same way. Then the intestine was cut open, doing short distances at a time, and the whole contents and mucous membrane were carefully scraped off, using the edge of a glass slide and collecting this material in a dish of water. The contents of the three dishes were then carefully searched through in small lots by means of a strong hand lens or a binocular microscope. On one occasion the head of a *Moniezia* was repeatedly thrown into the intestinal material in order to test the method, but it was quite easily found. This part of the work also proved negative, except in the one case mentioned, and in another two lambs of which one had a small cricket and the other an ant in the rumen.

Observations on Invertebrates.—In the beginning udder-washings were examined a few times, but it soon became apparent that the infection was very scarce and so the intention of examining udder-washings, soil, plants, etc., was given up. All the available time was then spent in making a survey of the invertebrate fauna of one camp that had always been heavily infected, and in disecting specimens after they had been allowed to feed on *Moniezia* eggs. The specimens were killed with chloroform and dissected fresh, all internal organs and the whole musculature, also of the legs, were examined. It was found that 12 different species had eaten the eggs, but no intermediate stage of the tapeworm developed in them. Some eggs were digested, while others passed through and out in the faeces, sometimes minus the shell, but usually the latter was intact. The invertebrates were allowed to feed on the eggs for at least 14 days before they were dissected, and the dissected material of those species that had eaten the eggs was drenched to two of the muzzled lambs, in order that any intermediate stage which might not have been recognized, and also the eggs which had passed through to the rectum and were now perhaps infective, could get a chance to develop further. All the cricket material was given to one lamb and the other species to the second lamb. The lambs were killed seven days after the last dose of this material, but no trace of infection was found.

The invertebrates were kept in large glass jars containing moist soil and a few dry clods on top, with grass plants growing in the soil. The jars were covered with muslin. Ripe segments of *M. expansa* were added daily except on a few occasions when none were available. In the case of the grasshoppers, the eggs were smeared on the grass and their ingestion was perhaps accidental. The other 11 species were all observed to eat the tapeworm material deliberately. The mites were found in a jar that had been used for several weeks. In this jar there had been no invertebrates that ate the tapeworm material, and the latter had become overgrown with fungi. In these small masses of fungus, mixed with numerous tapeworm eggs which still appeared normal, the mites were present in large numbers, and here also they were breeding. They, therefore, had ample chance of eating the eggs if they could. The ants were not kept in jars, but tapeworm material was placed under stones where they had their nests. There were two species of ants, a small black species, the common house-ant, and a large black species. These ants carried the proglottides into their nests. The woodlice were found under a stone amongst the big black species of ants.

The following is a list of the invertebrates found in the camp, with the result of feeding on *Moniezia* eggs:—

Invertebrate.	Number fed.	Eggs eaten by.	No eggs eaten by.
(*Carabidae*) *Harpalus cursorius*, Pér.	9	4	5
,, ,, *clavipes*, Boh.	2	2	—
,, ,, sp.	14	1	13
,, ,, sp.	7	2	5
,, 1 sp., not identified	1	1	—
,, 2 spp., not identified	2	—	2
(*Tenebrionidae*) *Trigonopus typhon*, Muls.	3	3	—
,, *Gonocephalum simplex*, F.	18	16	2
(*Histeridae*) *Triballus ascaphus*, Mars.	16	—	16
(*Ptinidae*) *Trigonogenius globulus*, Sol.	1	—	1
(*Cydnidae*) *Cydnus lepidus*, St.	1	—	1
(*Chrysomelidae*) *Ageniosa* sp.	7	—	7
(*Crioceridae*) *Lema bilineata*, Germ.	1	—	1
(*Reduviidae*) *Coranus* sp.	2	—	2
,, ? ,, sp., not identified	4	2	2
(*Coleoptera*) not identified	1	—	
,, larvae, not identified (?*Harpalus*)	22	10	12
(*Collembola*)	7	—	7
(*Chilopoda*), not identified	7	—	7
(Earthworms) *Fridericia pretoriana*, Steph.	20	—	20
(*Acarina*) *Amolops* sp., *Glycyphagus* s	58	—	58
(*Gryllidae*) *Liogryllus bimaculatus*, De Geer ,, *Gryllus* sp.	15	14	1
(Spiders) 6 spp., not identified	14	—	14
(*Hemiptera*) 2 spp., not identified	3	—	3
(*Diplopoda*) not identified	25	20	5
Acrididae) *Ochrophlebia ligniola*, Serv.	5	2	3
,, 4 spp., not identified	12	—	12
(*Lepidoptera*) caterpillar, not identified	1	—	1
Ant, small black, not identified	23	—	23
,, large ,, ,, ,,	29	—	29
(*Woodlice*)	14	—	14
43 Species	344	77 (12 spp.)	267

The invertebrates were determined and classified by the Imperial Bureau of Entomology, for which I would like to express my gratitude. Some could not be determined, while of others no material was sent, as only the few specimens that were dissected could be found.

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APPENDIX.

While this article was in the press a publication by Daubney ("The Important Parasitic Worms of Sheep in Kenya," *Kenya Dept. Agric., Bull. No. 6 of 1928*, Govt. Printer, Nairobi, 1929) was seen, in which Daubney states that he took ten newly-born lambs from a clean area on to an infected area and fed them on cow's milk. Five were muzzled. Within five months the five unmuzzled lambs developed heavy infestations, while the five muzzled lambs remained free up to the age of eight months, when they were destroyed.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

Physaloptera canis, n. sp.
A New Nematode Parasite of the Dog.

By H. O. MÖNNIG, B.A., Ph.D., B.V.Sc., Veterinary Research
Officer, Onderstepoort.

On the 28th May, 1929, a dog, bought from a native living in the vicinity of the Onderstepoort Laboratory, died of biliary fever and at post-mortem was found to harbour one male and two females of the worm described below, together with two young specimens of *Toxocara canis.*

The worms are fairly stout and white in colour. They were adhering to the mucous membrane of the stomach when found, but the membrane showed no lesions.

The cuticle is very finely striated transversely and has a number of large folds, especially anteriorly. It forms a prominent collar around the head (fig. 1). In the male and the one female the third fold is large and forms a second collar-like swelling behind the head, as shown in fig. 1, while in the second female the cuticle is dilated around the anterior end of the worm in a balloon-like fashion. As in *Physaloptera praeputialis* and *P. malayensis*, the hind ends of the bodies of all three specimens were retracted into prepuce-like sheaths formed by the cuticle (figs. 2 and 4).

The mouth is surrounded by the usual two large, lateral lips, each bearing two papillae and the external and internal teeth. The external tooth in the two females is short and blunt, .008 mm. high, while the internal trifid tooth is .019 mm. high and .039 mm. wide at its base. In the male the external tooth is not quite as blunt and reaches up to the height of the notches of the internal tooth.

The *male* is 30.5 mm. long and 1.1 mm. thick. The oesophagus has a short muscular portion, 0.7 mm. long, and a glandular portion 4.5 mm. in length; the whole oesophagus is therefore about 1/6th as long as the body. The nerve ring surrounds the muscular part of the oesophagus at a point 0.5 mm. from the anterior end of the body. The excretory pore and the cervical papillae are at the same level, 0.7 mm. from the anterior extremity.

The hind end of the male (fig. 4) is retracted into a prepuce-like sheath of the cuticle and is very much like that of *P. malayensis*, Ortlepp 1922. There are four, stout circumcloacal papillae which are stalked. The sessile papillae are three precloacals situated in a transverse row in front of the cloaca, two pairs in a transverse row behind the cloaca, two pairs near the middle of the tail and one pair at the end of the second third of the tail. The ventral surface of the hind end is roughened by strong ridges which run in a longitudinal direction, except for a number of circular ones surrounding the cloacal opening. In between these ridges a number of small thickenings are seen, especially along the median line.

The spicules are unequal; the left is 1.4 mm. long and the right 1 mm.; they are both fairly thick and strong, the left slightly thinner than the right.

The *females* are 44 mm. and 36 mm. long and 1.54 and 1.1 mm. thick respectively. It is remarkable that, in spite of this fair difference in size, in both females the muscular portion of the oesophagus is 0.8 mm. long and the glandular portion 5.5 mm.—the whole oesophagus, therefore, 1/7th and 1/6.7th respectively of the body length—and also the distances from the anterior end to the nerve ring, 0.6 mm., and to the cervical papillae and excretory pore, 1 mm., are equal in both. Also the anus is 0.68 mm. distant from the tail end in both, and the tail is retracted into the cuticular sheath (fig. 2).

In the larger female the vulva is situated 18.5 mm. from the anterior end, in the smaller female 14.6 mm., dividing the body length in the proportion of 1 : 1.4 and 1 : 1.5 respectively. The measurements of the internal genital organs and also their arrangement are exactly alike in both specimens. The vulva is inconspicuous and leads into the vagina, which runs forwards and is continued by a narrow ovejector, the whole being 2.9 mm. long. The end of the ovejector turns back sharply and opens into a wide egg chamber (fig. 3), which is 1.8 mm. long and is distended with eggs. From the posterior extremity of this chamber the two uteri originate like two horns, which both run forwards and are at first narrow. The one horn turns back soon after having passed the middle of the egg chamber, while the other reaches further forwards, widening out and turning back a short distance in front of the egg chamber. The eggs are small, thick-shelled, and only slightly ovoid; they are .043 mm. long and .033 mm. broad.

Host.—Canis familiaris.
Location.—Stomach.
Locality.—Pretoria.

Types in Onderstepoort Helminthological collection No. 2258.

Affinities.—In considering the identity of this worm three species of *Physaloptera* have to be thought of. *Physaloptera rara,* described by Hall and Wigdor in 1918 from the dog, on a single female which was perhaps not quite mature, although it was 24 mm. long, has a much shorter oesophagus than *P. canis,* the length being 1.16 mm. It was pointed out above that in both females of *P. canis,* which were not of the same length, the oesophagus is 6.3 mm. long, and the two species are therefore quite dissimilar in this respect. The vulva of *P. rara* is more anterior in position, the arrangement of the genital organs is different, and the tail has no prepuce-like cuticular sheath, although this may be due to immaturity. On the other hand, the teeth and the arrangements of the cuticle at the anterior end of the body are very similar in the two species. *Physaloptera praeputialis,* known from Felidae, has the prepuce-like sheath at the hind end of the body, but for the rest is quite unlike *P. canis,* except for the mode of origin of the uteri from the egg chamber. *Physaloptera malayensis* of wild Felidae and Canidae has the prepuce-like sheath, and the tail of the male corresponds closely to that of *P. canis*, while also the horn-like origin of the uteri from the egg chamber is found in this species. However, the cuticle is very slightly reflected over the lips and is not at all voluminous anteriorly, the external tooth is as high as the internal, the ratio of the lengths of the two spicules is quite different, and there are minor differences in other measurements. *P. canis*, therefore, appears to be a valid species, and since it appears to be normal and is closely related to *P. malayensis*, a parasite of related carnivora, it is probably a true parasite of the dog. The host animal was bought 18 days before its death, and during this period had no opportunity of eating another animal that could have been the host of the parasite.

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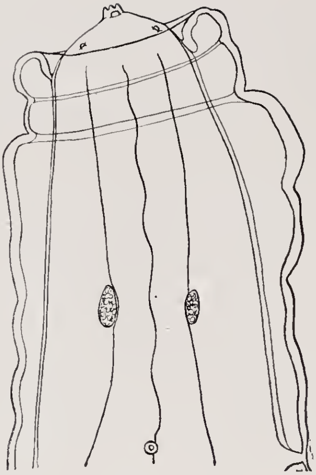

FIG. 1.
Physaloptera canis—anterior end.

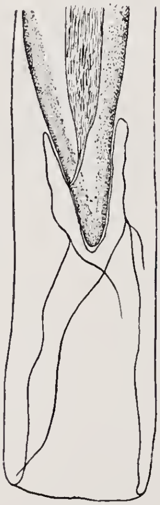

FIG. 2.
Physaloptera canis—hind end of female.

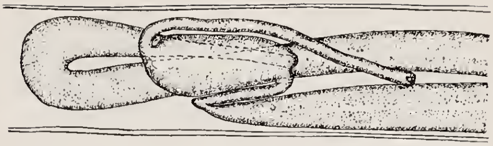

FIG. 3.
Physaloptera canis—female genitalia.

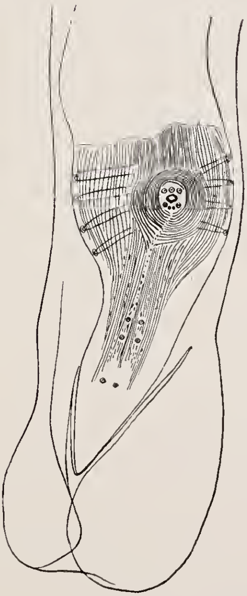

FIG. 4.
Physaloptera canis—hind end of male.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

On the Morphology of a Schistosome *(Schistosoma mattheei,* sp. nov.) from the Sheep in the Cape Province.

By F. VEGLIA, Dr.Med.Vet. (Turin), late Veterinary Research Officer, Onderstepoort;
and
P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In 1926, Mr. J. I. Edgar, the Government Veterinary Officer at Port Elizabeth, sent the Director of Veterinary Education and Research a few schistosomes collected from the mesenteric veins of sheep in the Humansdorp District. The material which was submitted to Veglia for identification was rendered practically useless by its poor state of preservation. Veglia visited the infected farm and collected material. The examination of this proved the parasite to be closely related to *Schistosoma bovis* (Sonsino) Khalil 1924. More material was subsequently collected and examined by Le Roux, who also recovered eggs from the faeces of bovines on the infected farm.

As the parasite differed in some important details from *S. bovis* as described by Khalil, it was thought advisable to obtain specimens of *S. bovis* from Italy and Egypt.

Dr. Khalil kindly sent us two males from Egypt. Veglia's (1928) appeal for specimens from Italy was unproductive, as was the attempt to obtain specimens of *S. bovis* from Dr. Cawston in Durban.

Having failed to obtain the desired material, it was decided to accept Khalil's (1924) description of *S. bovis* as that of the parasite recovered by Sonsino from cattle and sheep in Egypt.

Sonsino's description of the parasite is not available in South Africa, and we had to accept the description as quoted by others.

The South African species for which the name *Schistosoma mattheei*, sp. nov., is designated can be differentiated from *S. bovis* (Sonsino) Khalil 1924, both morphologically and by the pathological lesions provoked in sheep.

MORPHOLOGY.

The parasites collected from the portal vein, its tributaries, and its hepatic ramifications were preserved in 10 per cent. formalin in some cases and in 70 per cent. alcohol in others, after washing in physiological saline.

MALE CHARACTERS.

The males, which are white in colour, were readily observed in the mesenteric veins or in the blood collected from the portal vein.

Specimens, dropped alive into the preservative, appeared with the anterior portion, bearing the suckers relatively short, and straight (Fig. 1), or flexed dorsally at the ventral sucker. In other specimens the anterior sucker was directed ventrally (Fig. 2).

In the males, allowed to die in physiological saline or in the case of specimens allowed to die in the veins prior to collection, the anterior portion was comparatively straight.

The different measurements of both males and females were affected by the modes of preservation adopted. In specimens similarly treated and measured the same structures showed variations indicating that certain measurements are of little or no generic importance.

Dropping live parasites into the preserving fluid produced strongly contracted specimens. These were difficult to handle for obtaining accurate measurements.

The measurements given below are of specimens preserved in 70 per cent. alcohol or 10 per cent. formalin after allowing them to die in normal saline.

The males measure 17 to 22 mm. in length. The average was 18 to 19.5 mm. With the lateral edges folded in, the maximum diameter was about 1 mm., but in flattened-out specimens this was practically doubled.

The *anterior sucker* with a lateral diameter of 0.4 mm. was placed obliquely, facing antero-ventrally. The inner surface of its strongly muscular wall is lined with strong spines. These spines were prominent on the buccal surface of the dorsal portion, projecting anteriorly beyond the ventral one.

The *ventral sucker*, placed approximately 1 mm. behind the anterior sucker, is either fully (Fig. 1) or partially extruded or wholly retracted into the body (Fig. 2). When fully extruded its lateral diameter, slightly more than its antero-posterior, is about 0.5 to 0.6 mm. Its ventral surface is lined with spines.

Genitalia.—These consist on an average of four to six *testes*, antero-posteriorly compressed (Figs. 1 and 2), a *seminal vesicle* anterior to the testes, and a short ejaculatory duct which opens in the middle line, posterior to the ventral sucker and at the beginning of the *gynaecophorus canal*.

This canal, formed by the ventrally folded-in lateral edges, begins about 0.5 mm. behind the ventral sucker and runs the full length of the worm.

Its inner surface is armed with spines, while the *dorsum* or outer aspect of this portion of the body is studded with numerous cuticular tubercles armed with spines.

The portion of the body carrying the armed suckers is smooth and unarmed.

The alimentary canal begins anteriorly as a continuation of the funnel-shaped depression of the anterior sucker; it continues posteriorly to bifurcate on reaching the ventral sucker. The two intestinal caeca do not reunite (Fig. 4), or, if they do, only shortly before the posterior extremity is reached. They bifurcate again into two short caeca, one shorter than the other (Fig. 5). In some specimens the intestinal caeca have been observed to reunite and bifurcate three to four times before ending in a single caecum (Fig. 6) or in two short caeca.

The excretory system does not differ from that described for other schistosomes.

The Female Characters.

The females are cylindrical in shape, with the ovary situated about the junction of the anterior and posterior halves. The average length of the females varied between 17 and 25 mm. They were of a dark colour imparted to them by the dark intestinal contents. From the ovary the worm tapers anteriorly. Posteriorly practically no diminution in breadth is discernible until the very extremity is reached.

The *anterior* and the *ventral suckers* are much reduced compared with those of the male, but, like them, are armed with spines. The rest of the body is smooth and unarmed.

The *alimentary canal* bifurcates immediately in front of the ventral sucker into the caeca, these reunite on reaching the caudal extremity of the ovary and continue as a common caecum to within 0.1 mm. of the posterior extremity.

The *genitalia* comprise the usual organs met with in trematodes. The vulva, situated immediately behind the ventral sucker, leads into a *uterus* measuring 8-9 mm. in length, and containing from five to ninety-five ova. It terminates posteriorly at the *shell gland*, which is an ill-defined mass of tissue lying in front of the ovary. Into it passes the *oviduct* from the caudal extremity of the ovary.

The *common vitelline duct*, formed by the union of the two main lateral ducts, posterior to the ovary, accompanies the oviduct past the ovary, and then seems to fuse with it on reaching the shell gland.

An *ovary*, measuring approximately 1 mm. in length with a maximum diameter of about 0.2 mm., lies partially between the intestinal caeca, immediately in front of their reunion.

The *vitelline glands* surround the posterior common caecum and occupy practically the whole posterior half of the worm (Fig. 1). In most specimens they stop only within 0.19 mm. of the posterior extremity. The *excretory pore* is subterminal.

The eggs in the uterus measure from 92 μ × 40 μ to 170 μ × 44 μ, while the eggs passed in the faeces measured 170 μ × 72 μ to 280 μ × 84 μ. The average in the latter group was 180 - 240 μ × 60 - 80 μ.

The eggs in a preserved portion of the caecum measured 160 μ × 60 μ to 200 μ × 80 μ.

There is no doubt that the ova continue to increase in size after being deposited in the venules.

TABLE

The Chief Measurements of the Females

Authors		Khalil (1924)	Various authors quoted by Montgomery (1906)
Locality		Egypt	Egypt and Italy
Species		*S. bovis*	*S. bovis*
Hosts		Cattle and sheep	Cattle and sheep
Habitat		Veins, alimentary tract and uro-genital organs	Veins, alimentary tract and uro-genital organs
Length of worm		12–17 mm.	15–20 mm.
Length of the common caecum		3–4 mm.	12–14 mm.
Eggs passed in		Faeces and urine	Faeces and urine
Measurements of the eggs	In utero	160–180μ × 50–60μ	160–180μ × 40–50μ
	In gut-wall		
	In faeces		

No. 1.

of S. mattheei, *sp. nov., and Related Species.*

Neumann and Meyer (1914)	Montgomery (1906)...	Bertolini (1908)......	Veglia and Le Roux.
Egypt...............	India................	Italy................	Cape Province.
S. bovis.............	*S. indicum*..........	A variety of *S. bovis*	*S. mattheei*, sp. nov.
Cattle and sheep.....	Equines and sheep...	Cattle...............	Cattle and sheep.
—	Veins, alimentary tract	Veins, alimentary tract	Veins, alimentary tract.
13–18 mm.	10–17 mm. (Average 13·5 mm.)	20–27 mm.	17–25 mm.
—	5·5–6·5 mm.	14 mm.	8–12 mm.
—	Faeces..............	Faeces...............	Faeces.
150–160μ × 38μ 240 × 60μ	Immature 100–115μ × 44–48μ Mature 125–136μ × 53–60μ	132–165μ × 30–44μ 181–198μ × 39–49μ 214–247μ × 53–59μ	92–170μ × 40–44μ 160–200μ × 60–80μ 180–240μ × 60–80μ

The ova passed in the faeces may be oval or fusiform. The spine is well developed and terminal.

From the description of this species it would appear to be related to *Schistosomum indicum* Montgomery 1906, *Schistosoma bovis* (Sonsino) Khalil 1924, and *Bilharzia crassa* (Sonsino) Bertolini 1908.

The different measurements given for *Schistosoma bovis* (Sonsino) by various authors are given in Table 1, together with those of *S. indicum* and *S. mattheei*, sp. nov.

From these data it is evident that the species described in this article is distinct from *S. indicum* and *S. bovis* (Sonsino) Khalil 1924, but probably identical with the species recorded from Italy and held by Bertolini to be a *variety* of *S. bovis* (Sonsino).

As regards the pathological lesions observed, the new species has never been found invading the urino-genital organs nor producing lesions as recorded for *S. bovis* by Sonsino, Khalil, and others. *Bilharzia crassa* of Bertolini behaves in this respect as *S. mattheei*, sp. nov., and is probably identical with it. The re-examination of the Italian bovine and ovine schistosome is therefore required.

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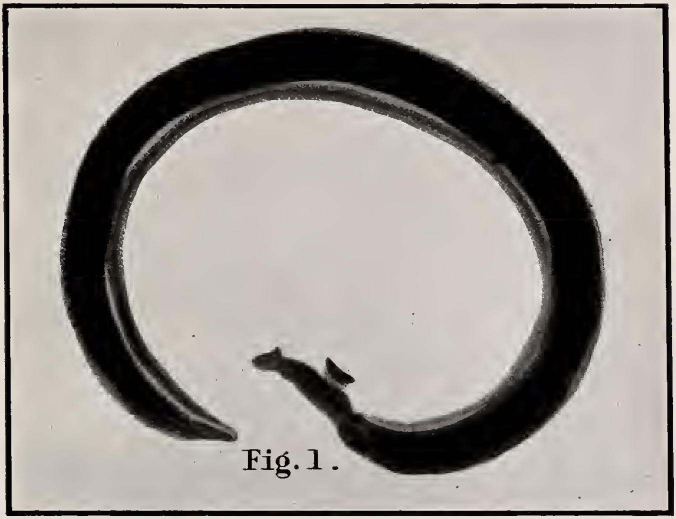


Fig. 1.

A male of *Schistosoma matthei* sp. nov., with the ventral sucker extruded. Magn. 14 ×.

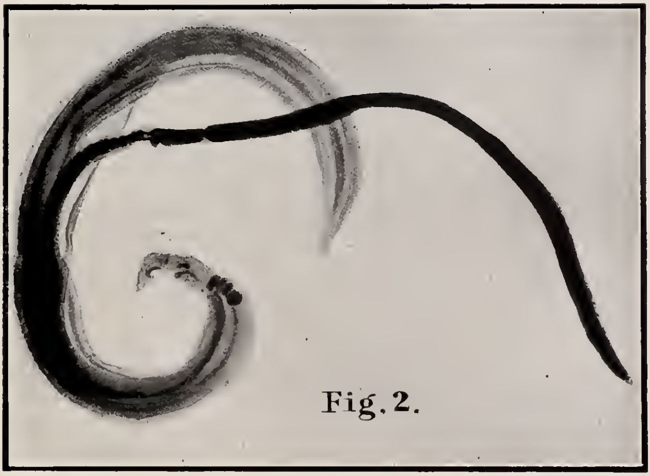


Fig. 2.

A male of *Schistosoma mattheei* sp. nov., with the anterior extremity of the female clasped in the gynaecophorus groove. Magn. 13 ×.

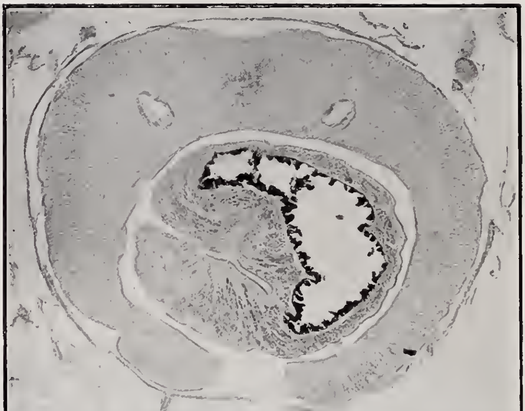


Fig. 3.

Transverse section of a male with a female in the gynaecophorus groove. Female sectioned posterior to the union of the intestinal caeca. Parasites in a vein. Magn. 75 ×.

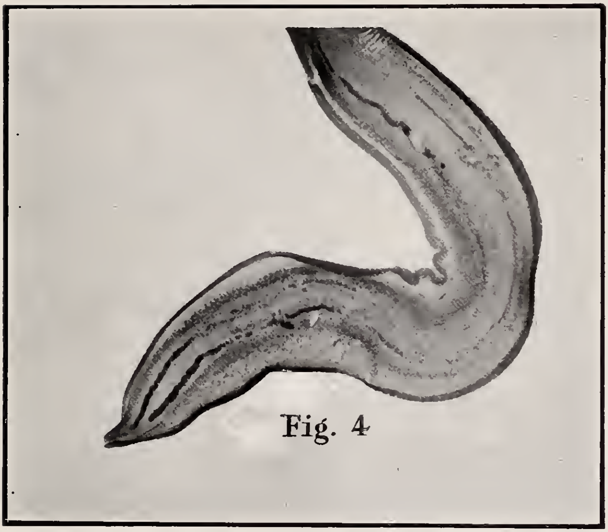
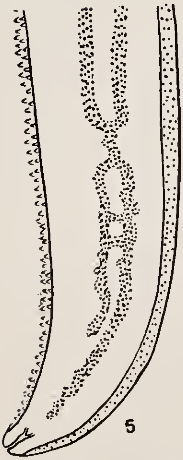


Fig. 4



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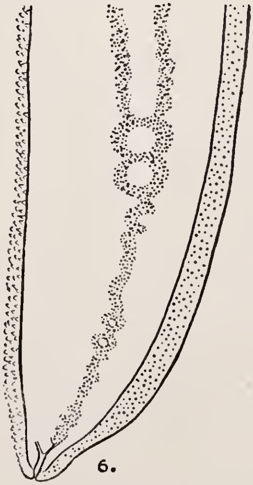
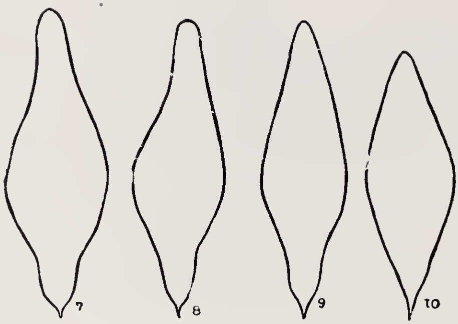
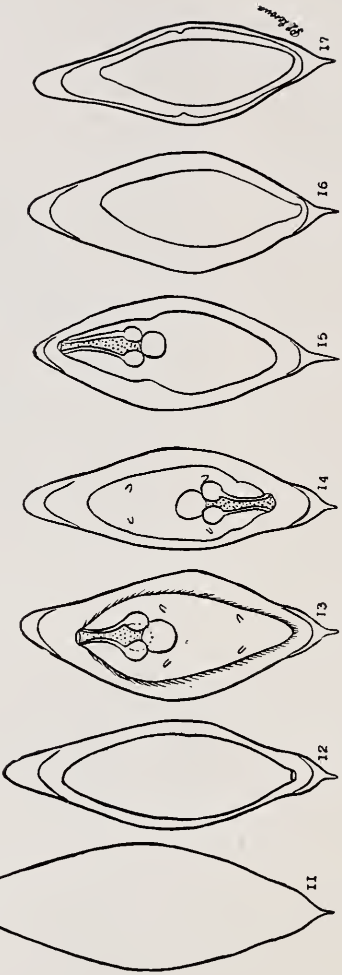
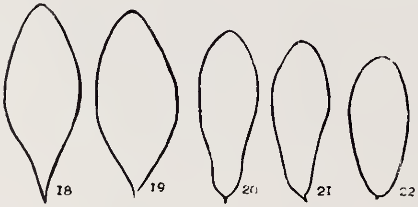


6.

Figs 4-6.—Variation in the termination of the intestinal caeca.







Figs. 7–10.—Eggs deposited in the wall of the caecum.
Figs. 11–17.—Eggs from the faeces.
Figs. 18–22.—Eggs from the uteri of two females.

*15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.*

Remarks on the Habits and the Pathogenesis of *Schistosoma mattheei,* together with Notes on the Pathological Lesions observed in Infested Sheep.

By P. L. LE ROUX, B.Sc (Edin.), M.R.C.V.S., Veterinary
Research Officer, Onderstepoort.

INTRODUCTION.

The prevalence of Schistosomiasis as an ovine malady of Merinos in the Cape Province was first recognized in 1926 when Mr. Edgar, the Government Veterinary Officer then stationed at Port Elizabeth, was questioned by a young farmer, Mr. S. W. Matthee (L.'s son), about peculiar white objects seen in the mesenteric veins of his dying sheep.

Before describing the habits of *S. mattheei* Veglia and Le Roux, 1929, in the definitive host or recording the pathological lesions provoked by this species, it seems desirable to review briefly the lesions attributed to certain schistosomes parasitizing cattle and sheep.

Although the pathological lesions associated with the three common human schistosomes have received the attention of a host of investigators and have been dealt with in innumerable publications, the pathology of the analogous disease in domestic stock has been sorely neglected.

It is surprising that, although schistosomiasis of domestic animals has been known since 1876 and is widespread, pathologists have little availed themselves of the opportunity of studying the analogous disease in domestic stock. A comparative study would undoubtedly have resulted in the elucidation of various points, associated with human schistosomiasis, which have puzzled man for years. Experimentation with domestic stock would have facilitated and permitted experiments to which man cannot be submitted.

Fairley (1920), utilizing monkeys for investigating some of the more puzzling points, made interesting observations. His experiments have added to our knowledge of the lesions provoked. The same author four years later used goats to test the efficacy of tartar emetic and emetine hydrochloride against *S. spindalis.*

The smaller laboratory animals, and even the domestic fowl, have within recent years been utilized in determining the life-cycles of the common human schistosomes. Although the cat and the dog are very suitable experimental animals for the study of the pathology and habits of *S. japonicum*, I have been unable to trace much literature in this connection.

OBSERVATIONS ON THE HABITS OF "S. MATTHEEI."

As some of the observations on the behaviour of *S. mattheei* in the definitive host are at variance with those recorded for other schistosomes, I propose dealing with my findings in detail.

THE DISTRIBUTION OF THE PARASITE IN THE SHEEP.

The pathological lesions produced by schistosome infestation have been shown by various investigators to be closely related to the veins invaded.

The habitat of *S. mattheei* was found to be influenced by certain factors and it is accordingly considered advisable to discuss the distribution of the parasite in the definitive host as observed in sheep killed for autopsy, in sheep killed after treatment, and in sheep dead from natural causes.

(*a*) *The Distribution of the Parasite in Naturally Infected Sheep killed for Autopsy.*

In the majority of sheep killed for autopsy the adult schistosomes were most abundant in the smaller veins draining the small intestine and the caecum. They were less frequent in the larger radicles of the portal vein, the portal vein itself, and its hepatic ramifications.

In animals not heavily infected, schistosomes were rather infrequent in the hepatic branches of the portal vein. This predilection for the small intestine and the caecum accounts for the heavy deposits of ova in the submucosa and the mucosa of these parts.

The invasion of the veins draining the *rumen*, *reticulum*, *omasum*, *abomasum*, *pancreas*, *colon*, *rectum*, and *mesenteric lymphatic glands* was as a rule very light. Nevertheless, a careful search revealed the presence of specimens. The *splenic vein* was very seldom invaded. In none of these cases were schistosomes observed in the veins draining the urino-genital organs. A careful search of the posterior vena cava and its pelvic branches, the cavities of the heart, and the pulmonary arteries and veins failed to reveal any parasites.

(*b*) *The Distribution of the Parasites in Animals which had received Intravenous Injections of Tartar Emetic, Emetine Hydrochloride and Sb.* 212.

That the administration of the drugs mentioned caused the affected parasites to migrate from their normal habitations was evident from the finding of abnormally small schistosomes in great numbers in the portal vein and its hepatic branches and in fair numbers in the right heart, the posterior vena cava, and the pulmonary arteries and their sub-divisions.

No parasites were recovered from the left heart, the coronary arteries, the pulmonary veins, the aorta and its branches, nor in the veins of the urino-genital tract.

(*c*) *The Distribution of the Parasites in Animals dead from Intercurrent Diseases.*

The observations were made on animals which had died from heartwater on the infected farm, and on animals which had died shortly after arrival at this Institute. The infected animals stood the 820 miles railway journey lasting four to five days very badly.

The distribution of the schistosomes in animals which had succumbed after a prolonged period of *decubitus* showed a variation from the two groups described above. In these cases there was a decrease of parasites in the venules and smaller veins draining the small intestine and caecum, but a marked increase of parasites in the larger tributaries of the portal vein. The blood-clots from the portal vein and its hepatic branches contained schistosomes in great abundance. In most of these animals schistosomes were present in fair numbers in the right ventricle and auricle and the pulmonary arteries and their ramifications. None were present in the left heart, pulmonary veins, or the veins of the urino-genital organs.

CONCLUSION AND DISCUSSION.

From the observations on these three groups it is evident that normally the adult *S. mattheei* inhabits the tributaries of the portal vein, but that adverse conditions cause the parasite to migrate into other localities.

Probably in the case of the dying animal the toxins diffusing into the bloodstream from the decomposing, stagnant alimentary contents, cause the parasites to forsake their normal habitat in search of a more congenial one elsewhere. With the administration of drugs lethal to schistosomes the same results were obtained.

Although it has been assumed (Fairley, 1920) that schistosomes can reach the pulmonary arteries via the haemorrhoidal plexus, this has not to my knowledge been confirmed.

Lampe (1927) is of the opinion that, in cases of heavy infestations, some cercariae may remain in the pulmonary arteries and complete their development there. He further states: " My opinion, based upon pathological-anatomical alterations in the lungs of bilharzia patients, is that full-grown parasites in the lungs need not be rare." He records a case where he squeezed a few full-grown *S. mansoni* from the pulmonary vessels. He unfortunately does not specify whether these were from the arteries or the veins nor does he specify whether the observations were made on a human who had succumbed prior or subsequent to the administration of a drug lethal to schistosomes.

Turner (1909) records adult schistosomes (*S. haematobium*) from the pulmonary vessels of natives who died on the gold mines in Johannesburg. Houghton (1923) records *S. japonicum* from the pulmonary arteries of man.

The presence of adult schistosomes in the lungs of man and animals has been recorded by only a very few investigators.

OBSERVATIONS ON THE MIGRATION OF OVA.

Fairley (1920) discusses in detail the penetration of the wall of the venule and the passage of the ova of *S. mansoni* and *S. haematobium* into the perivenous tissue. According to him, the spines of the deposited ova engage the vessel wall and pierce it. The force of the venous bloodstream drives the ova through the wall of the venule. I fail to see how the force of the venous bloodstream alone can accomplish this. Should the rupture of the venules and the migration of the ova through the tissues into the lumen of the gut not be attributed partly to the peristalsis of the intestines? During slight constipation, ova and bloodstained mucus, containing ova in clumps, were more abundant than with softer faeces. This seems to indicate that the migration and liberation of ova deposited in the latter part of the large intestine is greatly influenced by the passage of rather firm faeces along the alimentary tract.

Fairley (1920) describes the passage of the ova from the perivenous tissue into the lumen of the gut as follows: "The spines," after piercing the wall of the venules, "take no part in the process of extrusion of the ova through the tissues to the exterior, which is dependent on the inflammatory softening and cellular accumulation in its vicinity."

This statement cannot be accepted. The pathological changes observed in sheep and guinea-pigs disprove it.

Dew (1923) expresses views similar to those of Fairley. It seems unlikely that the ova deposited in the intestine of man and monkeys should behave differently from those in sheep and guinea-pigs.

In Byam and Archibald (1923) we read that the ova progress through the tissues by elaborating a toxin and that the ova consequently become surrounded by eosinophiles, polymorphonuclears, and small mononuclears. The progress is therefore attributed to a process of ulceration.

According to Hutchison (1928), the escape of the ova from the mucosa is accomplished by a process of ulceration, described as follows: "An intense inflammatory reaction occurs with the accumulation of many leucocytes, lymphocytes, and endothelial cells. Among the leucocytes is a large number of eosinophiles. Irritation of the mucosa and the loss of its normal resistance to bacterial invasion result in actual abscess formation. The abscesses are as a rule very superficial. As they rupture into the lumen of the intestine or bladder, they discharge ova together with their contents. Granulations spring up at once in the ulcerated areas and may become excessive. As they grow they continue to carry ova up through the mucosa to the surface. From the fact that adult parasites are commonly found in the loose areolar tissue under such areas of ulceration and granulation tissues, and from the large number of ova in such a place, it seems likely that the gravid female is attracted to areas where the processes of tissue repair are active."

It seems most unfortunate that such conclusions should have been arrived at from the study of material collected from heavily infested humans where death had probably intervened only after a protracted period of *decubitus*. It seems most unlikely that the material examined was obtained from subjects which had succumbed from pure infections of schistosomes. It is a well-recognized fact that the type of Egyptian on which post-mortems are allowed is of the working class. He is not uncommonly a subject of malnutrition and not infrequently heavily infested with a host of endo-parasites, protozoal as well as metazoal.

My experience with sheep is that only those free of other diseases affecting the alimentary tract, and killed for autopsy, are suitable subjects in which to study the migration of the ova through the tissues into the lumen of the gut.

THE MIGRATION OF THE SCHISTOSOMES IN THE BLOOD-VESSELS.

Fairley (1920), recording his observations on *S. haematobium* and *S. mansoni*, states that progression within the veins is by peristaltic-like movements of the body and by the action of the ventral sucker.

In the case of *S. mattheei* progression is by a rather interesting cycle of somatic movements, depending on the calibre of the invaded blood-vessels.

In the larger veins, with the wall not contracting on the body of the parasite, progression is essentially, if not wholly, by prolongation and contraction of the portion of the body situated anterior to the ventral sucker, aided by the two suckers acting as organs of fixation. The parasite proceeds as follows: It anchors itself to the wall of the vein by means of its ventral sucker armed with spines; the portion anterior to the ventral sucker is protruded, and whilst fully extended, the armed convex ventral surface of the projecting dorsal portion of the anterior sucker is firmly affixed to the vessel wall; the ventral sucker is released and the protruded portion contracts. Considerable ground is thus covered with each cycle of movements completed.

Progression in the smaller veins, distended by the parasite, is somewhat modified. The posterior portion of the body is now also actively utilized. Since the anterior portion can no longer drag along the rest of the body, we find that the massive posterior portion forces itself along by a series of somatic prolongations and contractions. The well-developed layers of muscles are now brought into play. The cycle of events here is somewhat as follows: A portion contracts, forming a circular swelling distending the enveloping vein still further and fixes the parasite, while the immediately anterior portion is protruded along the vessel: a swelling as described above now forms at the end of the extended portion. This enables the posterior portion of the parasite to be drawn forward.

Several of these circular swellings may be observed to appear simultaneously along the body. The retreat of the parasite from an invaded and distended venule is undoubtedly achieved in like manner, but in the reverse direction.

The cuticular tubercles, armed with spines, on the external (dorsal) surface of the male are undoubtedly adaptations for facilitating and accelerating progress. That their presence is not absolutely essential for migration is proved by their absence in *S. japonicum*, a species parasitizing man, his domestic stock, and other mammals in the Far East.

The presence of a *dorsal groove*, not hitherto described for male schistosomes, may now be recorded. This groove, extending from the shoulders posteriorly, was not only evident in transverse sections of the parasite in tissues, but also in the fresh and in the preserved specimens. Anteriorly it is deep, but becomes more shallow posteriorly. That one of the human schistosomes possesses a similar groove is evident from Hutchison's (1928) figure 3, plate 2. It is quite probably an adaptation to prevent total arrest of the venous flow in the invaded veins. Complete stagnation of blood in a vein would undoubtedly affect the parasite adversely.

Fairley (1920) and Dew (1923) state that the females of *S. haematobium* and *S. mansoni* leave the males, when the time for oviposition arrives, to invade the smaller venules of the selected organs. This desertion of males was not manifested by the females of *S. mattheei*. Males still partially embracing the females were often encountered in the submucosa of the invaded alimentary tract. I have, hitherto, not yet encountered females, unaccompanied by males, in the wall of the digestive tube.

It seems quite unnecessary that females, in search of venules in which to oviposit, should abandon the males. The arrangement of the venules within the wall of the gut is such that numerous venules, suitable for oviposition, are within easy reach. In the many cases examined the female does not necessarily continue in the vein selected by the male, but was almost without exception found with her anterior half invading a small collateral tributary of the vein holding the male.

The progression of the female within the venules is, as in the males, accomplished by a series of somatic movements, in which the anterior and ventral suckers and the intervening portion of the body play a not insignificant rôle. The mode of retrogression from the invaded venule appears to be accomplished by a contraction of the body, commencing at its attachment to the male, and by traction applied by the male. It would, therefore, appear essential that a portion of the female be permanently clasped by the male.

Most of the pairs encountered in the large veins appeared with a short length of the caudal extremity of the female trailing behind and with the cephalic portion just protruding from the anterior limit of the *gynaecophorus groove*. In others the female is wholly imprisoned in this groove and eggs are occasionally deposited here.

Whether the spines in the *gynaecophorus groove* allow the male the recovery, actively or passively, of the ovipositing female is not definitely settled. That muscles control their direction is evident, but whether they are actively or passively utilized to allow the partial escape and the total recovery of the female is still to be solved.

It seems possible that the female, uterus laden with eggs (seventy to ninety is a common number), migrates into a venule. As she is withdrawn by the male, the venule, closely contracting on her body, forces the eggs out. The presence of forty or more eggs lying in a row and partly overlapping each other seems to favour this mode of oviposition more than that outlined by Fairley (1920). The uterus having been emptied of most of its eggs, the female withdraws or is withdrawn into the *gynaecophorus groove* of the male to feed, and produce more eggs. The fact that parasites were never encountered singly, in sheep destroyed for autopsy, seems to indicate that schistosomes are normally monogamous. That separation, by accident, must occasionally occur is to be expected.

In animals which had succumbed after prolonged *decubitus* and in animals subjected prior to destruction to drug-treatment, single males and females were occasionally observed in the larger tributaries and hepatic branches of the portal vein and in the pulmonary arteries.

In none of the naturally infected sheep were males more frequent than females, as recorded for other schistosomes [Montgomery (1906) and Fairley (1920)]. These authors based their finding on parasites collected solely from the portal vein and its hepatic branches.

The females, owing to their dark colour, are not so easily detected in the clotted blood as are the stouter and lighter coloured males. Montgomery examined animals which had either died of *Surra*, in the case of equines, or of *Gillar*, in the case of sheep. In the case of the monkeys and the goats artificially infected by Fairley, the predominance of males may be attributed to: (i) that more male than female producing cercariae [Cort (1921), Faust (1927), and Severinghaus (1928)] were administered; or (ii) that prior to death, or as the result of treatment with drugs, the males became separated from the females, which, for reasons given above, were not so readily detected.

THE PATHOLOGICAL LESIONS RECORDED FOR SOME OF THE KNOWN ANIMAL SCHISTOSOMES.

In 1876 Sonsino recovered a schistosome from the portal vein of a bull at Zagazig in Egypt. On account of the peculiar shape of the ova, he regarded it as a new species and named it *Bilharzia bovis.* On recovering it the following year from the portal veins of sheep, he changed the name to *Bilharzia crassa.* No matter how unsuitable, the former name has preference.

Having been unsuccessful in obtaining Sonsino's original communication describing the lesions observed in infested sheep and cattle, I shall quote his findings as translated by Fleming (1892) from Neumann (1888). These read:

> " In an ox, the blood of which furnished thirty of these parasites, Sonsino observed very pronounced intestinal catarrh, with thickening, exudation, and ecchymoses in the walls of that tube. The vesical mucous membrane was ecchymosed and covered with papilliform elevations, which were yellow at the point and about the size of a pin's head. The vessels contained the ova of Bilharzia."

In the same paragraph the author continues: " It is possible that the haematuria which so frequently affects cattle in East Africa and the Cape of Good Hope is due to this parasite." It is not quite clear whether this last quotation should be attributed to Sonsino or Neumann himself.

Neumann's (1888) statement:

> " Le *B. crassa* détermine chez les animaux des désordres analogues à ceux qu'engendre chez l'homme le *B. haematobia* "

has undoubtedly influenced many subsequent authors of textbooks on Veterinary Parasitology and Pathology.

Stiles' (1898) statement:

> " The worm is said to bring about in cattle and sheep the same lesions of the bladder, intestine, etc., which *S. haematobium* causes in man "

has apparently influenced American veterinary literature. According to Stiles (1898), two transport bullocks suspected of being affected with rinderpest were destroyed in Calcutta. From the anus of one, Bomford removed papillomatous growths harbouring ova resembling those of *S. haematobium.* In the mucosa and submucosa of the caecum from the other he observed numerous ova.

Kaupp (1918), discussing "*Schistosomum bovis*," writes:—

> "The flukes are found principally inhabiting the veins of the abdomen, more especially the venous plexuses of the bladder and rectum. They may cause nephritis, cystitis, and as a result bloody urine or faeces. The adjacent lymph glands become hypertrophied. The condition may terminate in death."

Underhill (1920), in a textbook on Veterinary Parasitology, considers under the heading " Bilharziosis " only *Schistosoma bovis* and mentions haematuria, painful micturition, bloodstained faeces, and " a condition somewhat resembling piles " as the chief symptoms of schistosomiasis in cattle and sheep. According to this author, most animals being only lightly infected, show a slight chronic cystitis, whereas heavily infected beasts may succumb from rupture of the urinary bladder or from uraemia, sequelae to acute nephritis.

Piller (in Hoare, 1915), in his chapter on " Trematodes of the Ox," writes:—

> " Schistosomiasis or bilharziosis is manifested chiefly by symptoms appertaining to the bladder. These are due to the eggs, and not the adult parasites. Haematuria is usually the first alteration in health; then there is pain in the loins and urino-genital tract."

Henschen (see Joest, 1923) writes:—

> " Von Pathogen Trematoden der Blase kommt bei den Haustieren eigentlich nur eine Art, *Schistosomum crassum* vor. Er wurde hauptsächlich in den Mittel-meerländern gefunden, besonders beim Rind, weniger oft beim Pferd und Schaf. Der erwachsene Parasit lebt im Pfortadersystem und gelangt von dort in die Venen des Darms und der Blase. Aus den Gefässen der Blasenwand kann er ins Blasenlumen eindringen, wonach er entweder hier liegen bleibt oder mit dem Harn gleich ausgespült wird. In der Harnblase entwickeln sich geschwürige Prozesse (Marotel), die selbt zur Perforation führen können (Piot-Bey). Oft nimmt der Prozess einen mehr produktiven Charakter an, wobei kleine warzenförmige Neubildungen entstehen."

Woolridge (1923), under " Bilharziosis," writes:—

> " The immature stages of various species of Bilharzia worm inhabit the portal veins of the liver. When numerous they may give rise to enlargement and cirrhosis of the liver with the development of white plaques of fibrous connective tissue surrounding the portal vessels. The lesions result chiefly from the irritation by the eggs laid by the parasite, and partly to toxins they elaborate. Clinically the principal symptoms are those of dysentery, due to a similar deposition of eggs in the finer branches of the portal veins in the walls of the intestine, especially the rectum."

The following passages from Ceradini's review of Bertolini's (1908) article are well worth quoting:—

> " niemals aber konnte bei den sardinischen Rindern die Bilharziose der Blase wahrgenommen werden."
>
> " Die Veränderungen des Dunnsdarms kommen bei der Bilharziose der sardinischen Rinder durch katarrhalische Enteritis und Knotchenbildung in der Submucosa zum Ausdruck. Die Veränderungen des Mastdarms sind viel weniger stark ausgesprochen und betehen hauptsächlich in kleinen punktförmigen Blutungen der Schleimhaut."

> "Die Knotchenbildungen in der Submucosa des Dünndarms werden durch *Bilharziaeier* und junge Exemplare des incystierten Wurms hervorgerufen, haben aber durchaus nichts zu tun mit den hyperplastischen Formen und den polypenförmigen adenomatösen Vegetationen, die *Bilharzia* Mastdarmenzundung beim Menschen kennzeichnen."
>
> "Der erwachsene Parasit wählt sich mit Vorliebe das Pfortadersystem zum Sitze aus und ganz besondere dessen hepatische abzweigungen, sowie die Venen des Dünndarms."

In Chopra and Chandler (1928) we read:—

> "Schistosomes of several species, of which the commonest is *S. bovis*, live in the mesenteric blood-vessels; as in the case of human schistosome infections, severe mechanical injury is caused by the eggs to the walls of the bladder and rectum, but much is still to be learned as to the degree to which cattle suffer from these infections. The life-cycles of these worms is similar to that of the schistosomes of man. Schistosomes infect sheep and goats, and in experimental animals have been shown to be injurious."

Marotel (1927) reports on the pathogenesis of *Schistosomum bomfordi* as follaws:—

> "Schistosomose bovine, se traduisant par une entérite, avec *ecchymoses* punctiformes, *ulcérations* hémorragiques, *végétations* polypeuse; outre ces altérations dues aux oeufs, on observe encore des *nodules* sous-muqueux développés autour des femelles contenues dans les veinules."

v. Ostertag (1923) reports on Schistosomiasis of cattle as follows:

> "Manche der mit Schistosomiasis behafteten Rinder leiden an Haematurie; bei der Schlachtung findet man die Parasiten (dauernd das Weibchen vom Mannchen umschlungen) in den Hinterleibsvenen, ferner chronische Enteritis mit nekrotischen Herden in Darme, in denen meistens die Eier des Schistosomum finden, und Knotchen (mit den Larven des Schistosomum) im Dunndarm."

In the following words Khalil (1924) refers to the pathogenesis of *Schistosoma bovis*:—

> "The disease affects cattle and sheep, causing lesions similar to those in human bilharziosis. In a fair number of the cases reported, although the adult parasites were found, no lesions or only slight partly congestion of the lower part of the large intestine was found."
>
> "Cirrhosis of the liver is caused by *S. bovis* infection, just as in human infection especially with *S. mansoni*; but cirrhosis in cattle is common and is caused by a multitude of parasites, a common cause in Egypt being *Fasciola gigantica*."

He further comments on the suspicions of various observers that *S. bovis* may be the cause of haematuria in cattle in East Africa and the Cape. He is, however, inclined to ascribe this haemoglobinuria to *Piroplasmosis*. He does not mention the existence of *Piroplasmosis* in Egypt.

He records the Cape as an endemic area for *S. bovis*, but unfortunately omits to quote his authority. In an extensive hunt for references, I have failed to trace any communication recording the recovery of schistosomes from cattle and sheep in the Union of South Africa. Mönnig (1928) lists *S. bovis* as having been found in the heart and blood-vessels of cattle, especially in Natal. He does not quote his authority. I still believe that this article contains the first record of schistosome ova having been recovered from the faeces of cattle in the Union of South Africa. The ova were identical with those from the infected sheep on the same farm.

Faust's (1926) statement: "In fact, the 'cow' hypothecated by Dr. Cawston may not be necessarily involved in the *Schistosoma bovis* life-cycle in Natal; man may possibly serve in this capacity," is based on a statement from Cawston (1920) that he has not yet traced the cow, the usual host of *Schistosoma bovis*.

Montgomery (1906) records the finding of schostosomes in the portal veins of two sheep from Bareilly near Lahore. No macroscopic pathological lesions were, however, seen nor were ova found. He admits, though, that a careful examination was made only of the rectum and urinary system. These schistosomes he evidently regarded as *S. bovis*. He next mentions that Baldrey had recovered from sheep schistosome ova of the human type (presumably, ova shaped like those of *S. haematobium*) and closely resembling those of *S. indicum*.

Montgomery finally recovered from the portal system of two other sheep adult schistosomes indistinguishable from *S. indicum*. He observes that there were no lesions beyond "small areas of discrete punctiform haemorrhages present throughout the length of the small and large intestines."

Baldrey (1906), in his discussion of *Gillar*, records having found eleven of the fifteen Gillar affected sheep infested with schistosomes resembling those recovered by Montgomery from the sheep, horse, and donkey.

According to Baldrey, the lesions attributable to this parasite were only evident in the small and large intestines, noticeably so in the duodenum. He records a muco-enteritis, with the mucosa necrotic and probably shedding off in places. Here and in the mucosa of the rectum schistosome ova could be demonstrated. The liver, spleen, kidneys, pancreas, lungs, and heart were not affected. He described changes, observed in the mesenteric and omental fat, which indicate fat necrosis.

Gillar is undoubtedly a malady provoked by a heavy panverminosis in association with a decrease in the amount of nutritious foods available in the natural pastures during the lean seasons.

The immature amphistome, mentioned by Baldrey in his article, is in the Union of South Africa occasionally responsible for heavy mortality amongst sheep and cattle, and was probably the cause of the lesions attributed by him to *S. indicum*.

According to Lee (1927), *S. indicum* would appear to be a comparatively benign haematozoal parasite of camels in India.

Hall (1923 ?) states that *Schistosoma bovis* inhabits the veins of both the rectum and the bladder and that the eggs may therefore be passed in the urine and in the faeces.

Fairley and Mackie (1925), dealing with the pathology of *Schistosomum spindalis* in goats, state that the lesions are most closely related to those of *S. mansoni* and *S. japonicum*, which it resembles in habitat. According to them, the verminous ante-mortem thrombosis in the portal or mesenteric veins and its branches and the hepatic lesions, varying from the early bilharzial pseudo-tubercles to a "pipe-stem" periportal cirrhosis, are as common as with *S. japonicum* in man. They further observe that the spleen is seldom affected and that bilharzial papillomatosis was not present owing to the non-irritating character of the ova. The absence of any inflammatory reaction around the deposited eggs of *S. japonicum* is likewise attributed to the lack of an irritant. It seems most remarkable to assume that the ova of different schistosomes should progress through the tissue by different means and that some species should oviposit ova possessed of an irritant not present in those from other species.

THE PATHOLOGICAL LESIONS OBSERVED IN THE INFESTED SHEEP.

Apart from the presence of the parasites in the mesenteric veins, macroscopic lesions were evident in the lungs, the liver, the walls of the small and large intestines, the periportal lymphatic glands, and in the pancreas.

On opening the thoracic and abdominal cavities, the macroscopic lesions manifested varied with the age and the degree of infection and with the condition of the animal.

On exposing the abdominal contents, the first lesion to strike the eye was the presence or absence of fat in the greater omentum and mesentery. In a large percentage of cases there was an average amount of fat present. This fat invariably showed multiple fat necrosis. The absence of fat was associated with a slight hydropericardium and hydrothorax.

In the mesenteric blood-vessels the schistosomes could be demonstrated by holding a loop of gut up against the light with the mesentery still attached to the abdominal wall. In animals killed for autopsy the migration of the parasites within the veins was studied in the mesentery thus suspended.

For enumerating the number of parasites present it was absolutely essential to ligature the portal vein close to the liver and remove the intestines by severing the mesentery along its dorsal attachment. The blood from the liver and the portal vein was run into a shallow flat-bottomed circular glass dish, and the parasites collected.

When the usual post-mortem routine practised at this Institute was followed and the mesentery severed along its alimentary attachment, many parasites were lost and damaged; the collection was rendered much more tedious and difficult, and a true estimation of the number of worms present was impossible.

THE SMALL INTESTINE.

The small intestine showed fine grey specks scattered diffusely throughout its wall. The microscopic examination of a small portion of the wall flattened between glass slides showed that the pigmentation was due to the presence of numerous schistosome ova deposited in clumps or singly. A high percentage of these ova was calcified. Parasites were present in the submucosal and subperitoneal veins. Small haemorrhages in the mucosa propria were often numerous.

The caecum appeared with wall thickened. On holding the organ, incised along its mesenteric attachment, up against the light, certain areas, chiefly localized along the greater curvature, did not transmit light. (Figs. 13 and 14.) At these spots the wall was much thickened, the mucosa was sandy-coloured and emitted a grating sound when cut with a sharp broad-bladed post-mortem knife, showing that calcareous material was present. The cut surfaces showed the submucosa much thickened, pigmented, and fibrosed. Running the finger tips across these areas felt like passing them over fine sandpaper.

The colon and rectum showed less deposition of ova within their walls, as could be expected from the small percentage of schistosomes observed in their veins.

The presence of small haemorrhages from minute vessels was observed in the mucosa propria of the large intestine.

A microscopic examination of these revealed the presence of numerous schistosome ova, calcified or containing active miracidia.

The contents of this portion of the alimentary canal were in the majority of cases of normal consistence, but covered with bloodstained mucus. The microscopic examination of this mucus revealed the presence of small clots of blood holding schistosome ova. Most of the ova contained very active *miracidia*. In a few cases there was such an abundance of sticky mucus that the hard faecal pellets, lying more or less in a single row, could on section of the gut be removed *en masse*. Mucus, enveloping the pellets. was often observed hanging in a string from the anus. That the passage of firm faecal matter facilitates the liberation of ova from the mucosa must be accepted. It was frequently observed that attacks of constipation were followed by diarrhoea, the diarrhoea being quite probably a sequel to the invasion of the mutilated *mucosa propria* by bacteria.

No malignant or benign papillomatous growths as recorded for *S. bovis* in cattle and sheep (Khalil and Sonsino) or for *S. mansoni* and *S. haematobium* in man, were present in any one of the forty-five animals autopsied.

The microscopic appearances vary with the age of the infection.

In newly-invaded animals the ova lying in the venules (Fig. 29) were observed in fresh preparations made by scraping off the mucosa and examining it pressed between two glass slides. In sections, the ova were observed mainly in the *sub-mucosa* and passing through the *muscularis mucosa* into the *mucosa propria* (Fig. 23). In these various zones they were found surrounded by leucotypes (Fig. 35), especially small mononuclears, eosinophiles, and epithelial cells. The blood-vessels in the immediate neighbourhood appear slightly congested, and the mucous glands showed increased activity.

In cases of old standing, the most prominent lesion was the pronounced fibrosis of the wall of the digestive tube. The lesions observed were:—

(1) Schistosomes in pairs in the submucosa (Fig. 16), and occasionally in the subperitoneal veins.
(2) Pseudo-tubercles in different stages of development in the *mucosa propria* (Figs. 21, 23, and 24), in the *submucosa* (Figs. 22, 23, 24, and 25), and in the external and internal muscular layers (Figs. 21 and 22).
(3) Invasion and destruction of the *muscularis mucosa* (Figs. 21, 22, 23, and 24).

(4) Hyperaemia of the mucosa in some cases.
(5) Conspicuous absence of or a reduction in eosinophiles compared with those met with in the gut-wall of newly infected sheep.
(6) Hypertrophy of the walls of the veins.
(7) Increase of fibrous tissue in the invaded portions of the alimentary tract.
(8) The complete absence of tissues characterizing malignant growths.

CONCLUSIONS.

(i) No actual necrosis and erosions of the mucosa, as recorded from human cases infected with *S. haematobium*, *S. mansoni*, and *S. japonicum*, have hitherto been observed in sheep killed for autopsy. They were present in sheep that had succumbed after prolonged decubitus. It has already been suggested that some of the lesions attributed to schistosomiasis are secondary or attributable to bacterial, protozoal or metazoal invasion of the digestive tube.

(ii) The lesions observed in the alimentary tract of sheep prove that their production was stimulated by the presence of the parasites and their ova.

(iii) No malignant growths were encountered.

LIVER.

MACROSCOPIC APPEARANCE.

In cases of long standing the liver is decreased in size, of a shagreen appearance, and the surface may or may not be grossly lobulated (Fig. 3). In several cases the " hobnail " form of cirrhosis was observed. On palpation and on section the organ is extremely firm. The cut surface shows an increase of fibrous tissue in the portal tracts (Figs. 5 and 9). The cirrhosis is invariably of the multilobular type; occasionally areas showing monolobular cirrhosis were observed. The cut surfaces show dark pigmentation. In some cases a much lighter pigment in the perilobular tissues was discernible. The microscopic examination of fresh liver tissue showed that the latter pigmentation was due to deposited schistosome eggs.

In the more recently infected sheep the livers were markedly enlarged and of an abnormally pale colour; the consistence was slightly reduced; the surfaces smooth or nodular (Fig. 3); the cut surfaces showed the central veins open with the rest of the lobule somewhat opaque.

In a very recently infected lamb the liver showed a slight increase in size and numerous dull whitish specks under the capsule of both surfaces. Parasites were numerous in the portal vein and its ramifications.

Another type of liver met with was a heavily pigmented organ; increased in size with well-marked nodulation of both surfaces and with the consistence slightly reduced. In this type it would appear that the animal had become reinfected after cirrhosis; a sequel to a previous invasion had already been established.

In addition to these changes, the livers of some sheep showed calcified nodules of parasitic origin [*O. columbianum*, *Ecchinococcus granulosus* (cyst), and *Cysticercus tenuicollis*].

The more recent nodules due to *O. columbianum* were characterized by their greenish cheesy contents (eosinophiles accumulated in the vicinity of the migrating larva).

In the case of a ram which had died from intra-peritoneal haemorrhage of pancreatic origin, the bile-ducts appeared prominent owing to the thickening of their walls, stimulated by the presence of specimens of *Fasciola hepatica.*

In another case death followed as the result of a haemorrhage from the liver via the bile-duct into the duodenum. At post-mortem practically the whole of the small intestine was distended with coagulated blood.

In a ewe which had received intrajugular injections of a 6 per cent. solution of tartar emetic, the liver was enlarged and showed numerous emboli caused by dead schistosomes arrested in the hepatic ramifications of the portal vein.

Microscopic Appearances.

In the case of the atrophic cirrhotic liver, there is a marked increase of fibrous tissue in the portal tracts. The fibrosis is preceded by a cellular infiltration of these zones. Cellular accumulation is stimulated by the presence of the arrested ova (Figs. 5, 7, and 8).

Partially or completely organized thrombi (Fig. 6), practically obliterating veins, were frequently encountered. The walls of the viens show an increase of fibrous tissue. Pigment was as a rule present (i) in the immediate neighbourhood of the arrested ova, showing that it arrived there with the ova; (ii) in the lumen of the hepatic branches of the portal vein; (iii) in the *Stern cells* of Kupffner; and (iv) in the lumen of the central veins. It is undoubtedly via the liver that most of the pigment reaches the lungs.

Most of the eggs arrested in the liver showed calcification. In some cases (Fig. 5) the deposition of ova in the liver was heavy, but never so heavy as in the wall of the digestive tube (Figs. 13 and 14).

Fatty degeneration of the ova precedes calcification. *Giant cells* of the foreign body type were very seldom seen in the liver. Empty egg-shells were not encountered. Individual pseudo-tubercles were not so frequent as in the lungs and in the intestinal wall. In the recently invaded liver there were pseudo-tubercles showing different stages of development (Figs. 31, 32, and 33). The development of the pseudo-tubercle was observed to be as follows: an ova becomes arrested in a venule and may penetrate into the perivascular tissues; small nononuclears, endothelial cells, and eosinophiles surround it (Fig. 31); the included liver cells degenerate (Figs. 30, 32, and 33); eosinophiles which had penetrated to the egg perish there; fibroblasts begin and continue to develop until the necrotic mass is replaced by fibrous tissue and the egg is securely encapsulated and rendered harmless. Outside this developing capsule may occasionally be seen some eosinophiles, and small mononuclears and large endothelial cells containing pigment. Next to the egg may occasionally be observed a foreign-body giant cell (plasmodial mass). In no case was a giant cell found inside an egg-shell as recorded from human cases (Hutchison, 1928).

The structure of the pseudo-tubercle as seen in sections will vary with sections through it at different levels, and it is therefore best studied in serial preparations.

The microscopic examination of the liver of a sheep treated with tartar emetic showed that the emboli were due to dead schistosomes (Figs. 11 and 12). As in the lung (Fig. 25), the presence of these dead parasites stimulated cellular infiltration (Figs. 11 and 12).

The liver cells showed varying degrees of fatty degeneration. Degeneration was especially pronounced in cases of hypertrophic cirrhosis, but by no means uncommon in the atrophic form. The centre of the lobules often showed the liver cells deranged and degenerated, while the cells round the portal tracts appear unaffected (Fig. 35). There was an increase of connective tissue in the portal tracts. The chronic venous congestion of the liver contributed to the lesions referred to. The smaller bile-ducts were rather tortuous and showed proliferation of the endothelial cells.

Hepatic Cirrhosis.

According to most textbooks on Human Pathology, the liver in human schistosomiasis shows no contraction as in *alcoholic cirrhosis*. In some of the sheep autopsied there was a definite *polylobular* or *multilobular cirrhosis* and showing " hobnail " appearance (Fig. 3). In these cases the livers were enlarged and of a paler colour than normally. This slight yellowish discoloration was due to fatty changes.

That *atrophic cirrhosis*, *hypertrophic cirrhosis*, and intermediate forms were met with is proof that the one precedes the other. In a sheep infected for six months well-marked *hypertrophic cirrhosis* of the liver was encountered, while most of the old-standing cases showed a liver deeply pigmented, extremely firm, and with *atrophic cirrhosis* prominent. The livers, showing coarsely nodular bosses on the surfaces, were almost invariably hypertrophic. That these bosses result from the contraction of the fibrous tissue in an already enlarged liver is evident from the smooth-surfaced hypertrophic liver found in a six months infected sheep.

Taylor (1918), discussing atrophic and hypertrophic cirrhosis, mentions a case in which a liver reaching below the umbilicus, in a human with marked jaundice and no ascites, was found fifteen months later to have contracted quite close under the edge of the ribs.

In *malaria*, a protozoal disease characterized by the formation of a pigment, chemically indistinguishable from that produced by schistosomes, there is described a liver cirrhosis, monolobular in type and resembling the condition seen in alcoholic cirrhosis. Beattie and Dickson (1921) state that it is practically impossible, in any given case, to exclude all the other possible and well-recognized causes of cirrhosis and diagnose " Malarial cirrhosis."

Chronic hepatitis in domestic stock has been frequently recorded and has been ascribed to various causes. Observers have described cases where the *chronic hepatitis* in sheep, cattle, horses, and pigs was attributed to the migration of helminths.

Cysticercus tenuicollis and *Oesophagostomum columbianum* larvae (Figs. 43 and 44) in sheep; *Ecchinococcus granulosus* (cysts), *Fasciola hepatica*, and *F. gigantica* in sheep and cattle; *Strongylus*, *Habronema*, and *Parascaris equorum* larvae in equines; and *Ascaris* larvae in pigs, are in South Africa common causes of hepatic lesions.

Robertson (1906) in South Africa was able to produce atrophic cirrhosis of the liver in cattle and horses by feeding them repeated small quantities of *Senecio burchelli* and *S. latifolia*. Similar results were obtained by workers in North America and New Zealand with plants of the same genus. Other investigators suspect or have shown that toxins cointained in damaged (mouldy or rotten) food, toxius liberated during an attack of gastro-enteritis, toxins produced by helminths, bile stasis, chronic cholangioitis, and bacterial toxins and viruses are causal factors in the production of chronic interstitial hepatitis in our domestic stock.

Conclusions.

The changes in the livers of schistosome infested sheep should be attributed to:—

(1) the invasion by the developing schistosomes;
(2) the presence of eggs and exogenous pigment, of schistosome origin, arrested in the liver;
(3) the presence of dead adult schistosomes acting as emboli; and
(4) the chronic venous stasis.

That the cirrhosis may be partly due to a specific schistosome toxin cannot be completely ignored, but the present evidence seems to disprove its existence.

THE PANCREAS.

The pancreas in some cases showed a marked pigmentation of its more transparent portions. Pigmentation was due to deposited schistosome ova. In a number of cases the vessels were congested and contained schistosomes. In these cases the damage to the pancreas may have contributed to the fat necrosis observed.

Microscopically the pancreas showed marked changes from the normal. It was almost invariably involved. Eggs were recovered in all cases after the digestion of the organ in 4 to 5 per cent. caustic potash.

Sections revealed pseudo-tubercles, an increase in the amount of perilobular fibrous tissue, parasites in the veins, cellular accumulations around ova in the perilobular zones and those intralobularly located.

The Islets of Langerhans appeared normal. In heavily infected cases the walls of the veins were hypertrophied. Areas of the pancreas may appear degenerated (Fig. 36). In sheep the pancreas was undoubtedly more frequently involved than would appear to be the case in man. Dew (1923) observes that the pancreas is quite frequently involved in *S. mansoni* infections.

Judging from the habits of the different schistosomes, one would expect to find the pancreas more frequently invaded by *S. japonicum* and *S. mattheei* than by *S. mansoni*, and more often by the latter than by *S. haematobium*.

FAT NECROSIS.

According to Osler (1916), fat necrosis in the human ". . . occurs whenever the pancreatic juice, obstructed for any cause and dammed back on the gland, infiltrates its tissues, or escaping by the lymph spaces finds its way to structures at some distance from the gland."

He further observes that Balsar was the first to record fat necrosis in the interlobular pancreatic tissue, in the mesentery, in the omentum, in the abdominal fatty tissue generally, and occasionally in the pericardial and subcutaneous fat.

These observations of Balsar on the human also hold for the sheep. In sheep the pericardial, the subepicardial, and the subendocardial fat is not infrequently involved. Fat necrosis may be generalized without the pancreas being involved.

Opie (1901) states that fat necrosis is due to the fat-splitting ferment present in the pancreatic secretion.

It seems definitely accepted by the medical profession that fat necrosis is invariably associated with lesions in the pancreas. It has been stated to be most frequent in the acute forms of pancreatitis and less common in the suppurative forms.

Moir (1929) reports on a case of traumatic fat necrosis of the breast, and records: "The lesion differs from the fat necrosis of pancreatitis in being of slow evolution, so that phagocytosis of fat-cell disintegration products and fibroblastic proliferation keeps pace with cell destruction."

This type of fat necrosis has been observed in the omentum of sheep infested with *O. columbianum*. Here the trauma was induced by the larvae of the parasite reaching the omentum.

Farr (1923) procured fat necrosis of the subcutaneous fat in young pigs by pinching the fat with forceps.

Gottesman and Zemansky (cited by Moir) have met with cases of fat necrosis in the breast although there was no history of trauma. They, therefore, conclude that there may be factors other than trauma, and express the view that the decomposition of fatty material, escaped from a dilated duct, may stimulate the lesions.

Lee and Adair (1920) were evidently the first to associate trauma with fat necrosis in the human breast.

That fat necrosis is by no means uncommon in domestic animals is evident from Hutyra and Marek (1920), who record that Marek and Ronai had both observed lobular fat necrosis of the pancreas in absolutely normal lobules in fattened pigs, and that Mettam and Prettner had reported cases in dogs. It is recorded as of exceptional occurrence in the horse. In the horse we have one of the larger strongyles passing a stage of its life-cycle in the pancreas.

Cleland and Darnell-Smith (1912) point out that fat necrosis is amongst the conditions that may occasionally be mistaken for tuberculosis. They substitute the term *fat dissociation* for the term *fat necrosis* and record that in New South Wales it is of common occurrence in apparently healthy cattle and sheep. In sheep the lesions were smaller than in cattle, where they were usually present in the fat around the kidneys and in the mesentery and the omentum. " In cattle these areas may be single and an inch or more in diameter, or there may be several smaller ones, or they may be multiple. They seem usually located towards the centre of the fat involved, and a varying degree of fibrosis and puckering surrounds them."

They then record that this fat dissociation in cattle has not been found associated with any pancreatic lesions as has been observed in humans.

As regards the etiology of this condition, they cite two possible explanations, namely:—

> "According to one, the animal, as a consequence of luxuriant natural feeding, has stored up such an excessive amount of fat that the central areas of this substance are to all intents and purposes cut off entirely from the circulatory system, and are in much the same stage as the free fatty bodies mentioned as being sometimes found in the peritoneal cavity. . . . Under such conditions, presumably, the result would be autolysis of the cells, whose lymph supply has been diminished almost to the vanishing point, with the consequent appearance of fatty acid crystals within the cells, and the liberation of glycerine. It is to be noted in support of this view that the animals in which the condition occurs seem always to be in excellent health and remarkably fat.
>
> According to the other view, during some previous period in the life of a fat bullock, a demand has been made by the tissues for fat; the stimulus has reached the central areas of the various collections of fat, which by means of lipase have split up fat into fatty acids and glycerine. This having been accomplished, however, the poor vascular and lymphatic supplies of the more central portions of the fat masses have rendered it impossible for the fatty acids set free to pass into the general circulation. Better conditions for the animal then happening to occur, such as the passing away of a period of drought or the advent of much nutritious food, the demand of the tissues for fat has ceased, and the process of storage of fat has again proceeded, now forming a deposit around the central masses of dissociated fat, that is, in those parts of the adipose tissue better supplied with nutrient fluids. This would lead to a process of encapsulation of the dissociated fat; and the presence of the fatty acid crystals, acting as a foreign body, would result in the formation of a certain amount of fibrosis of the connective tissue in the neighbourhood."

From the views held by various workers it is apparent that fat necrosis in man and animals may be provoked by different etiological factors. The findings of Hariot (cited by Cleland and Darnell-Smith, 1912) surely explain why fat necrosis may occasionally be encountered throughout all fat deposited in the body. In the cases of traumatic fat necrosis of the breast, in the young pigs of Farr, and in cases associated with parasitic invasion of the omentum, the fat necrosis should probably be attributed to serum lipase, the serum being liberated by the injured tissues. The multiple fat necrosis noted by Balsar and observed in sheep, heavily parasitized by endo- or ecto-parasites, at this Institute can only be explained by the presence of a fat-splitting ferment in the general circulation.

Fat necrosis was a common lesion in sheep autopsied by me at Pietermaritzburg in Natal, at Ermelo in the eastern Transvaal, on farms in the western Transvaal, on farms in the Fraserburg, Worcester, and Humansdorp Districts of the Cape Province, at this Institute, and on a farm near Arlington Station in the Orange Free State.

De Kock (1928 *a*), referring to cases seen at this Institute, reports that he has found no lesions in the pancreas, and could not understand how the fat-splitting ferment could have reached the fat deposited outside the peritoneal cavity. He concludes with the statement: "At present the pathogenesis and aetiology of fat necrosis in the sheep are still to be regarded as unsolved."

In the various cases observed, fat necrosis was invariably associated with one or combinations of the following conditions: mild gastro-enteritis, gastric strongylosis (*Haemonchus cortortus*, *Trichostrongylus axei*, *Ostertagia circumcincta*); intestinal strongylosis (*Trichostrongylus instabilis*, *T. rugatus*, *Gaigeria pachyscelis*, *Bunostomum trigonocephalum*, *Cooperia curticei*, *Ostertagia trifurcata*, *Nematodirus spathiger*, *O. columbianum*); bronchial strongylosis (*Dictyocaulus filaria*); fascioliasis (*Fasciola hepatica*, *F. gigantica*); schistosomiasis (*Schistosoma mattheei*); ascariasis (*Psoroptes communis*, var. *ovis*, *Sarcoptes scabei*, var. *ovis*, *Ornithodorus mégnini*); myiasis (*Oestrus ovis*, *Lucilia sericata*, *Chrysomyia albiceps*); *Globidium* species in abomasum, pediculosis (*Linognathus stenopsis* and *Trichodectes caprae*) in goats, and in forms of icterus (bacterial and enzootic).

It has, however, by no means been proved that only the fat-splitting ferment from the pancreas is wholly responsible for fat necrosis. Fat necrosis is invariably found in anaemia associated with metazoal parasites (ecto- and endo-parasites). The presence of large areas of necrosed fat in the immediate neighbourhood of the pancreas seems to show that also in sheep a fat-splitting ferment may diffuse into the adjacent fat. Multiple fat necrosis and fatty degeneration of the liver were constant pathological lesions in sheep which had succumbed to a heavy artificial infection with *Haemonchus contortus* or *Psoroptes communis*. The lesions were especially conspicuous in those animals which were in good condition at the time of infection.

The stable diet of the experimental sheep at this Institute is whole or crushed maize and veld hay. The latter, being of poor quality, is not consumed to any large extent. The sheep exist practically on maize alone, fatten rapidly, and accumulate large quantities of abnormally oily fat. In addition to this diet, they received, if available, a certain amount of green feed: lucerne, green oats, green barley, barley wheat, or maize ensilage according to season.

The components of the ration are given, as it may play a rôle although this does not seem very likely.

In addition, it may be stated that the animals are confined to camps in the open or to rather small pens in stables, where they are not in direct sunlight.

These factors may contribute to the anaemia which ultimately induces or facilitates fat necrosis.

More details concerning the cause of fat necrosis in dogs, cats, and cattle should be looked for in countries where helminths invading the pancreas occur. This recalls a case in which I recovered two specimens of *Fasciola hepatica* from the pancreatic duct in a sheep. The occurrence of this trematode in this habitat has to the best of my knowledge not been recorded hitherto. They evidently migrated here via the bile-duct.

In some animals the fat necrosis was most intense in the fat which had been in contact with the rumen (Fig. 46). This would seem to indicate that the necrosis may be attributed either to a substance, diffusing through the wall of the rumen, or to the pressure of the rumen on the adjacent deposits of fat. That pressure or trauma may contribute to fat necrosis is supported by the presence of necrosis in the subcutaneous fat at the point where the sides of the feeding-troughs press against the throats of the feeding animals. Some of the troughs are uncomfortably deep and the grain at the bottom is not too readily secured.

The anaemia produced by ecto- and endo-parasitic infestation is almost invariably accompanied by multiple fat necrosis throughout the body and by a fatty liver. Fatty degeneration of the liver is common in sheep heavily infected with *Haemonchus contortus*, a very active blood-sucking parasite inhabiting the abomasum. The anaemia produced leads to defective oxidation and causes the " white liver," a term commonly used by the South African sheep farmer in describing a fatty liver Fat necrosis always accompanies this fatty liver of *haemonchosis* and usually precedes it.

Fat necrosis, associated with parasitic infestation in the sheep, seems to result from a too rapid dissociation of fat when the demand on the stored fat is made to meet the requirements of the body. The ferment utilized to effect the dissociation of the fat is probably liberated in too large amounts into the general circulation. The fat is dissociated into fatty acids and glycerine. The glycerine passes into the blood-stream, while the fatty acids are partly neutralized, forming soaps. A recent analysis of necrosed fat by Professor Malan at this Institute revealed that the calcium had increased by thirty-three times the amount present in normal fat. Calcium was also present as calcium carbonate.

The determination of the etiology of fat necrosis rests undoubtedly in the hands of the biochemist. The pathologist may postulate hypotheses, but the biochemist will have to prove or disprove them.

My observations on fat necrosis are recorded in the hope that they may stimulate further interest in a condition apparently not uncommon in man and beast.

THE SPLEEN.

The Spleen was in some cases slightly enlarged, with the malpighian corpuscles rather prominent. In a few cases the organ was slightly pigmented and its veins contained a few schistosomes.

Various investigators—Day and Ferguson (1909), Tsuchiya (1913), Day (1924 and 1925), Fairley (1920), Houghton (1923), Dye (1925), Hodson (1925), Christopherson (1925), Melency (1925), and others—consider splenomegaly as one of the lesions associated with human schistosomiasis. This splenomegaly most of them attribute to toxins liberated by the schistosomes.

Hutchison (1928) states : " Splenic enlargement is commonly, but not constantly, associated with bilharzia infections." Melency (1925) believes that splenomegaly results from the plugging of thousands of portal capillaries in the liver, followed by scarring and contraction of the tissues. The splenic enlargement was therefore merely the results of an obstructed portal circulation. Houghton (1923) observes that great splenic enlargement is a common lesion with heavy infestations. He cites portal stasis as the main cause, but does not deny that toxix products of parasitic origin may play a part.

Splenomegaly was rarely observed in the schistosome-infected sheep killed for autopsy. In an animal, killed a month after intrajugular injections with tartar emetic had ceased, the spleen was markedly enlarged, and macroscopic and microscopic examination showed the presence of numerous verminous emboli in the ramifications of the portal vein. This suggests the inadvisability of forcing the tartar emetic treatment by frequently repeated large doses.

Finally, it may be recorded that at this Institute there are hundreds of sheep autopsied annually and that splenomegaly is by no means confined to any one disease. In one victim it may be well marked, whereas in another victim of the same disease it may be absent. Splenomegaly is quite common in healthy sheep killed at abattoirs. With Enzootic Icterus, an ovine malady of undetermined etiology and characterized by haemoglobinuria and general icterus, I have in victims autopsied at Ermelo (1925), in the eastern Transvaal, and at the Pietermaritzburg Municipal Abattoirs (1925), constantly found a markedly enlarged spleen. Recoveries from the disease were, contrary to De Kock's (1928 *c*) findings, high.

LUNGS.

Macroscopic Appearance.

Macroscopically the most conspicuous and constant lesion met with in the lungs was their characteristic discoloration. The colour closely resembles that of the liver as painted by Dr. J. K. Lund [in Byam and Archibald (1923), Pl. 68, Fig. 1].

This discoloration of the lungs was observed in every animal, but varied in intensity with the age and the degree of infection. The pigment, causing this characteristic discoloration of the lungs, is the unassimilated portion of the blood ingested by the schistosomes. This unassimilated ingesta is returned per os into the general circulation and is removed from the circulation chiefly by the *Stern cells* of Kupffner in the liver. The remaining portion is removed by the connective tissue cells in the alveolar wall of the lungs and to a much less extent by the periportal, the bronchial, and the mesenteric lymphatic glands.

Most (if not all) of the pigment conveyed to the lungs appears to be phagocytized before the venous flow is gained. This probably accounts for the relative absence of pigment in sections of such organs as the heart, the kidneys, the skeletal musculature, and the central nervous system.

The extreme ventral and caudal edges of the lungs may appear free of pigment (Fig. 40).

Microscopic Appearance.

A constant microscopic lesion met with in sections of the lungs is the presence of pigment in the connective tissue cells of the alveolar wall. Another lesion which was fairly constantly observed was the presence of pseudo-tubercles next to bronchioles. These pseudo-tubercles result from the arrest of eggs in the arterioles. This is followed by a cellular infiltration resulting in emboli formation and the total obliteration of the arteriole. The final result is typical pseudo-tubercles (Figs. 26, 27, 28, and 29), which may partly reduce the lumen of the bronchiole and lead to secondary changes in the lungs.

In the developing pseudo-tubercle, pigment may, in the early stages, be found quite close to the arrested egg or eggs. In the fully developed ones the pigment is absent, having been removed by phagocytes. Foreign-body giant cells were only occasionally met with and appeared to be less frequent than has been observed in man.

The pleura were in a few cases found somewhat thickened, but whether this was solely due to schistosomiasis is not evident, as these animals were also suffering from a *verminous broncho-pneumonia.*

In the case of animals which had received intrajugular injections of drugs lethal to schistosomes, numerous parasites were found in the pulmonary arteries. These parasites were very much reduced in size, and the females harboured in their uteri rather small abnormally shaped eggs (Fig. 45).

The lungs of an animal which had received tartar emetic showed dead schistosomes arrested in the smaller arteries of the lung. The dead parasites stimulated cellular infiltration (Fig. 30).

Nasal discharges were examined for eggs, but none were ever found.

COMPARISON WITH THE LESIONS RECORDED FOR MAN.

The comparative absence (according to the literature) of lung lesions in man must be attributed to the lack of looking for them.

There are, however, a few references. Mackie (1885) and Bellili (1885) observed schistosome eggs in numerous small abscesses in the lungs. Eyles (1888), Bowlbey (1889), and Kartulis (1913) likewise record the presence of ova in the lungs. Symmers found a pair of worms in copula in the blood from the lung. Manson (1905) has observed ova in small numbers in the lungs, as did Pakes (Turner, 1908) in South Africa. Turner (1908) recorded lateral-spined ova from the lung of a Shangaan who had died of a nodular condition of the liver. This condition Turner attributed to either tuberculosis or syphilis. Out of 59 humans examined at post-mortem eggs were noted in the lungs of 37 and in the bladders of 52. In Byam and Archibald we have the lines: " Rarely in advanced and hyper-infected *S. haematobium* cases haemoptysis has been recorded with terminal-spined ova in the sputum." Dew (1923), discussing the lesions observed in the human lungs, records that in pure *S. mansoni* infection there is an almost complete absence of lung changes, in contrast to the lesions (pigmentation and patchy fibrosis) found in association with *S. haematobium* invasion. He suggests that the eggs reach the lungs either via the liver or through anastomatic channels between the portal and systemic systems.

Hutchison (1928) refers to the lungs in the brief statement: " Ova have been discovered in the lungs, brain, and kidneys."

With a progressive cirrhosis of the liver, the flow of blood through the organ is obstructed and the radicles of the portal vein become distended. The spleen is, therefore, often swollen. The increase of the pressure in the portal circulation is partly relieved by the widening of the anastomoses between the tributaries of the portal vein and those of the posterior vena cava. MacCallum (1919) points out that anastomoses between the portal and the systemic circulation are well described by Charcot (1882).

Conclusions.

Judging from the observations on sheep, it would appear that—

(1) the pathological lesions in the lungs are provoked by the presence of ova, pigment, and the parasites (migrating immature forms and dead adults);

(2) the lungs in *S. mattheei infection* are more often affected than with *S. mansoni* (a form of intestinal schistosomiasis) in man;

(3) the pigment which is not removed by the liver is removed by the lungs;

(4) the pigment and schistosomes may reach the lungs via the liver or via anastomoses widened as a result of the hepatic cirrhosis.

LYMPH GLANDS.

The most commonly affected lymph glands were the pancreatic, the hepatic, and the mesenteric. These were, as a rule, slightly enlarged and had the cortical zone heavily pigmented with a pigment chemically indistinguishable from the malarial pigment. The medulla of the mesenteric lymph glands often showed the presence of pseudotubercles. The presence of worms in these glands explains the presence of ova, while the pigment arrived here from the wall of the invaded intestine. The bronchial lymph glands were occasionally pigmented.

THE BLOOD CHANGES.

The marked eosinophilia recorded for schistosome-infected humans was not observed. This may perhaps be due to the fact that the disease was chronic. Several of the sheep were also infected with *Dictyocaulus filaria*, the only lungworm hitherto observed in South African sheep. The lungworm-infected sheep did not show an eosinophilia either, although portion of the lungs, on microscopical examination, showed heavy infiltration with eosinophiles. The bone-marrow did not show changes from the normal. This would lead one to deduct that the eosinophiles were formed from lymphocytes present at the lesion.

TOXINS EXCRETED BY SCHISTOSOMES.

According to Dew (1923), schistosomes " produce a toxin the reaction to which is manifested by eosinophilia and deviation of complement, and which is probably a big factor in the production of many of the pathological changes, especially those found in the liver."

Fairley (1920) states that the circulating toxins liberated by *S. mansoni* and *S. haematobium* are filtered out into the periportal zones. He attributes the generalized periportal cirrhosis of the liver to this toxin rather than to the presence of the ova.

Hutchison (1928) concludes that schistosomes produce " noxious substances " in the body of the host. According to him, " the nature of this noxious substance is not clearly established." He advances facts to prove that it results from simple protein splitting rather than from toxin production.

The presence of toxins liberated by the parasites themselves or by their ova has to my mind not been indisputably established. The fact that hyper-infected monkeys died does not necessarily prove the existence of toxins primarily produced by the schistosomes.

TOXINS ATTRIBUTED TO OTHER OVINE PARASITES.

There still seems to be a tendency to attribute the ill-effects elicited by helminths to " toxins " produced by them.

De Kock (1928), discussing *Haemonchus contortus* (the common stomach-worm) and *Oesophagostomum columbianum* (the nodular worm) infesting sheep, writes: " The worms live on blood (haemonchus) or produce poisonous substances (oesophagostomum)." This sweeping statement, unsupported by experimental data, is misleading and cannot be accepted, especially when Curtice (1890), the original discoverer of *O. columbianum*, states that the parasites are attached to the membrane of the large intestine and cause lesions in various organs.

Theiler (1921), in an admirable article on the ravages and lesions caused by the nodular worm of sheep, records the finding of worms attached to the mucous membrane of the large intestine. Mönnig (reprint from *Northern News*, Vryburg), discussing *O. columbianum*, writes: " The worms are not attached to the wall of the intestine, but lie mixed up with the contents." " These worms are not blood suckers." My personal observations support the findings of Curtice and Theiler.

The lesions observed in various organs (wall of small and large intestines, liver, omentum, mesenteric lymphatic glands, etc.) prove that the parasitic larval stage of this nematode is capable of inflicting considerable damage to tissues. There is no proof that the tissues, thus damaged, are not at some stage the producers of noxious substances.

That the migration of schistosome cercariae, adult schistosomes and their ova within the host, can damage the tissues is evident. That the damaged tissues cannot provoke the reactions observed has not been proved, but neither has it been disproved. The work of Lewis (1927) seems to support the view that stimulated or damaged tissues are capable of liberating irritants.

Geurrini (1912), dealing with the influence of trematode parasites on man, concludes that there is little evidence of toxin production. Several workers [Bedson (1913), Paulin (1914, Meyer (1913), Seyderhelm, and others] by injecting various worm extracts into experimental animals have provoked clinical symptoms or have induced pathological lesions in internal organs. These they attribute to toxins contained in the injected materials. They did not appear to have taken into consideration the nature of the substances injected.

With the advance of biochemistry this " Toxin theory " may one day be proved or disproved. In the meantime we have to accept Nicoll's (1922) lines: " Geurrini, on the other hand, does not appear to deny that pathogenic action may result from mechanical irritation, from the withdrawal of material essential to the host's economy, and from the promotion of secondary infection."

In South Africa the losses from the majority of gastro-intestinal parasites of sheep and cattle are seasonal and are most severe in a given locality when the natural grazing in that locality is at its worst.

The rapid disappearance of *Oesophagostomiasis* this spring from the flocks of the western Transvaal and Bechuanaland at the appearance of green pastures, after the first rains, proves to my mind that " the withdrawal of material essential to the host's economy " is a most important factor to be considered in the fight against parasitic diseases.

My personal observations are that sheep in good condition, kept on a well-balanced ration and free of other diseases, can only with difficulty be killed by infecting them with one species (*Dictyocaulus filaria*, *Schistosoma mattheei*, *Haemonchus contortus*, *Oesophagostomum columbianum*, or *Trichostrongylus*) at a time.

In some cases (*Dictyocaulus* and sheep, and *Haemonchus* and calves) it was impossible to infect animals in good condition. In others the infection was established only to be lost again within a short time. On reducing the rations of these animals they became infected. These infected animals were saved, provided they were not too heavily infected, by putting them back on to well-balanced rations. They increased in weight and lost the infection.

These experiments, conducted only with a few species, seem to indicate that animals in good condition possess the power of protecting themselves against certain helminths, and that infected animals when properly cared for can rid themselves of these worms.

In the case of *Schistosomiasis* my observations, based on field and laboratory experience, prove that infected sheep, on good pastures or on a balanced ration, relatively free from other helminths or ectoparasites and not unnecessarily worried, will not, during the productive period of their short span of life, die as the result of *S. mattheei* infestation. Records suggest that even in man schistosomiasis is a remarkably benign malady. The cases recorded as having been fatal within a few years occurred undoubtedly in malnutritioned, overworked, or otherwise debilitated individuals. Malnutritioned children, overworked, ill-clad, and underfed agricultural labourers, and already diseased subjects are likely to be very adversely affected by schistosome invasion. The successful control, prevention, and eradication of tropical diseases depend on better hygiene, less fatigue, and more suitable food.

That much of the injury caused by parasites is indirect, cannot be disputed. Do they unintentionally predispose their keepers' tissues to bacterial invasion or do they initiate this invasion as a defence against total extermination?

Galli-Valerio's (1914) remarks, that the attention paid to bacteriology has unduly distracted attention from the pathogenic propeties of worms, still hold to-day. We must, however, admit that the time and energy devoted to bacteriology has furnished us with some valuable data, to be applied in the investigation of the pathogenic properties of helminths.

If the future attempts at the control and the eradication of parasitic diseases are to be successful, there should be closer co-operation between Biochemists, Pathologists, Bacteriologists, and Parasitologists.

Other Helmints collected from the Sheep infected with Schistosomes.

In addition to *Schistosoma mattheei*, there were present (not necessarily all in any one animal) the species:—

Paramphistomum calicophorum in the rumen;
Fasciola hepatica (one case) in the bile and pancreatic ducts;
Haemonchus contortus, *Ostertagia circumcincta*, *Trichostrongylus axei*, and *T. instabilis* in the abomasum;
T. instabilis, *T. rugatus*, *Ostertagia furcata*, *Nematodirus spathiger*, *Bunostomum trigonocephalum*, *Strongyloides papillosus*, *Oesophagostomum columbianum* (nodules in gut-wall), *Avitellina centripunctata*, and *Moniezia expansa* in the small intestine;
Trichocephalus ovis, *O. columbianum*, in lumen of caecum and rest of large intestine;
O. columbianum nodules in gut-wall not too frequent;
Cysticercus tenuicollis in subperitoneum;
Echinococcus granulosus (cysts) in liver and lungs;
Dictyocaulus filaria in bronchi and bronchioles; and
Gongylonema scutatum in the mucosa of oesophagus.

The sheep which were dying were those simultaneously heavily infested with *H. contortus* and *Dictyocaulus filaria*. The former was successfully removed by the use of the Government Wireworm Remedy (a mixture of powdered copper suphate 80 per cent. and arsenic 20 per cent.).

In well-fed animals the lung-worm infection was lost within six to nine weeks after arrival at the laboratory. By keeping the animals on short rations the infection was retained.

Helminths collected from Small Antelopes shot in the Endemic Area.

One adult bushbuck (*Tragelaphus sylvaticus*) and three steenbucks (*Raphiceros campestris* Hunb.) were examined. The bushbuck yielded only three specimens of *Setaria* and from the steenbucks were collected only a few specimens of *H. contortus* and *T. rugatus*. The recovery of the latter from a wild ruminant, and its record hitherto from sheep in South Africa only, suggest that wild ruminants are the natural hosts.

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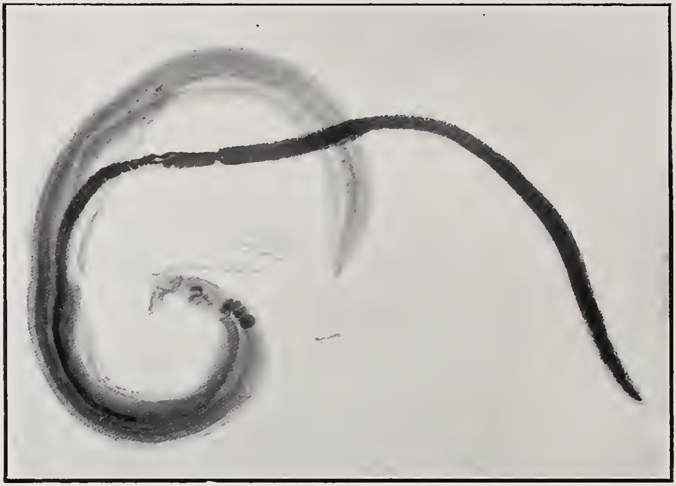

FIG. 1.
Schistosoma mattheei Veglia and Le Roux (1929). The female partly imbedded in the gynaecophorus groove of the male.

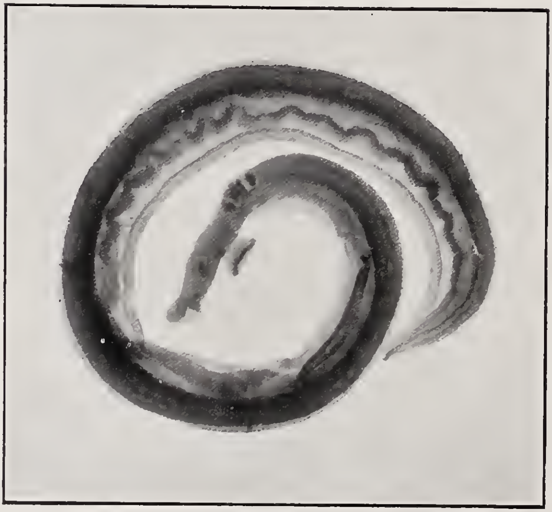

FIG. 2.
A male with prominent ventral sucker. Note the dorsal groove.

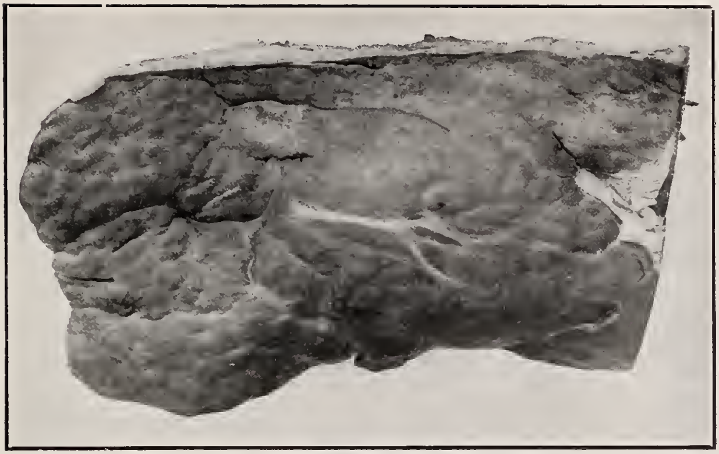

Fig. 3.

The visceral surface of a portion of a hypertrophic cirrhotic liver. Sheep killed.

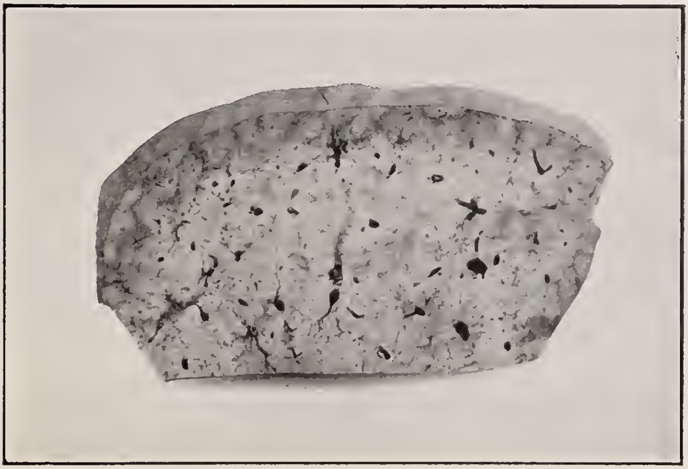

Fig. 4.

Portion of liver (case Fig. 3), showing pigmentation especially towards the periphery.

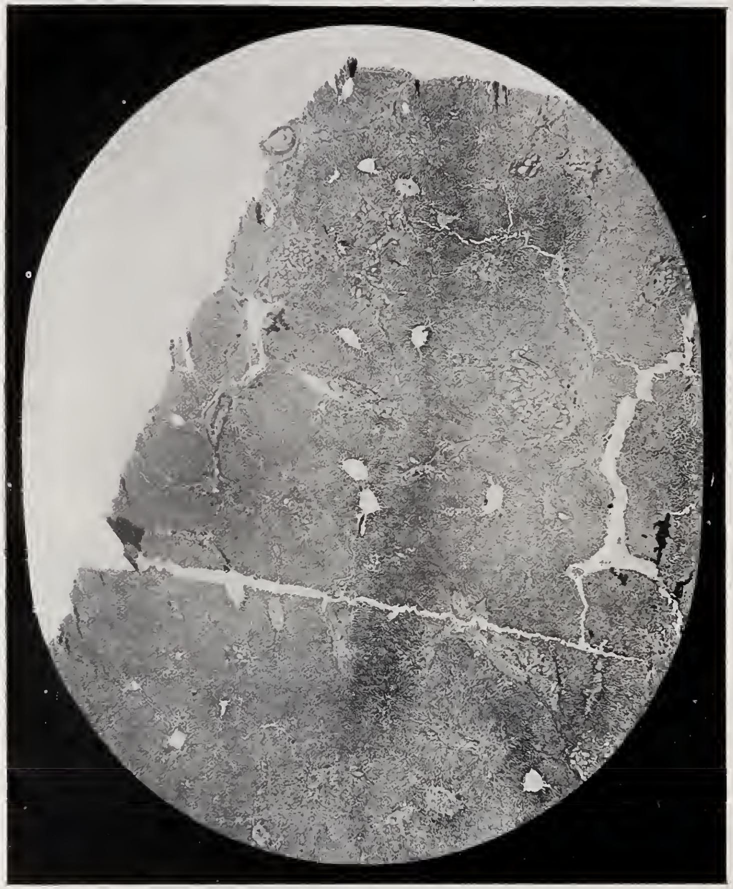

FIG. 5.
Encapsulated groups of eggs arrested in the liver. Sheep died.
Magn. 14 ×.

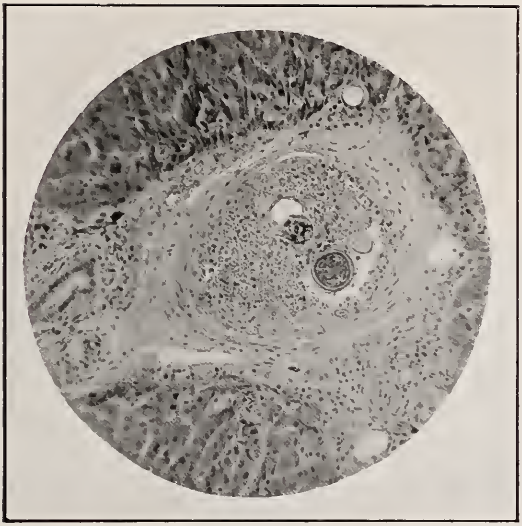

FIG. 6.

Two eggs and pigment in a hepatic branch of the portal vein. Sheep killed. Magn. 125 ×. Spec. No. 8324.

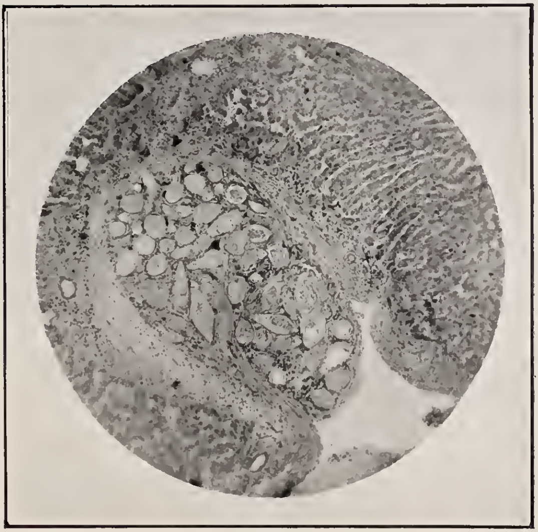

FIG. 7.

Eggs arrested in a branch of the portal vein. Sheep died. Magn. 60 ×. From Fig. 5. Spec. No. 8068.

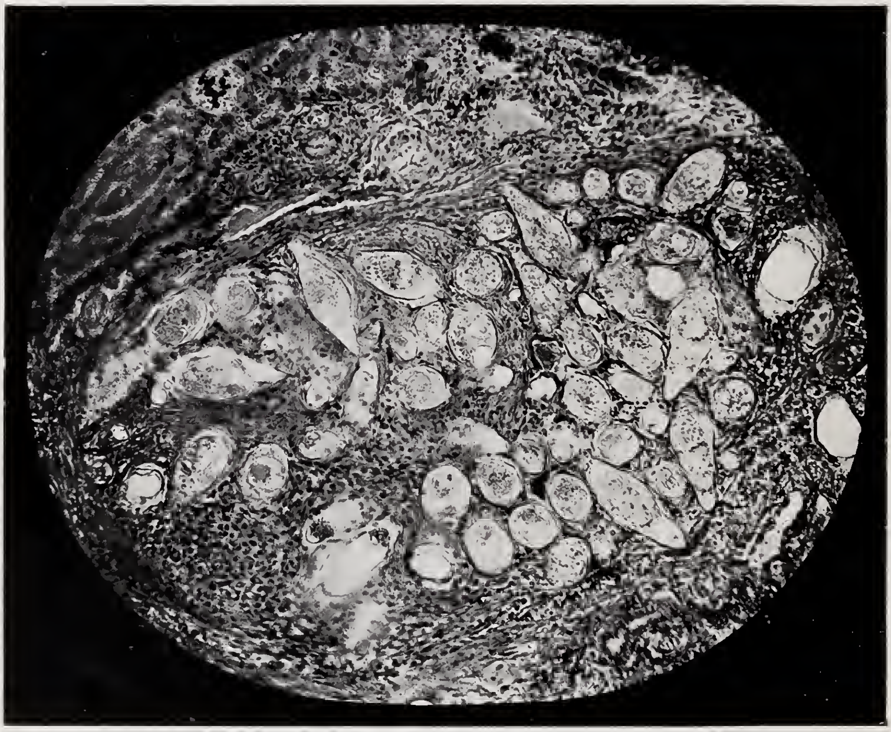

FIG. 8.

Encapsulated group of eggs in the periportal zone. Liver. Sheep died. Magn. 135 ×. From Fig. 5. Spec. No. 8068.

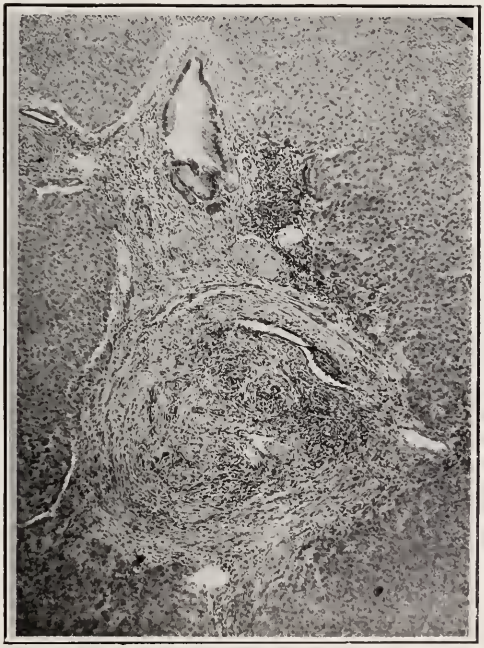

FIG. 9.

Hepatic branch of the portal vein, showing almost complete occlusion from an organized thrombus. Sheep killed. Magn. 70 ×. Spec. No. 8775.

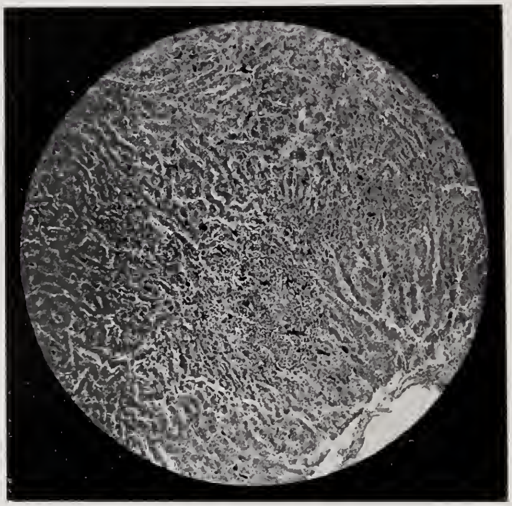

FIG. 10.
Degenerative changes and pigmentation. Liver. Sheep killed.
Magn. 75 ×.

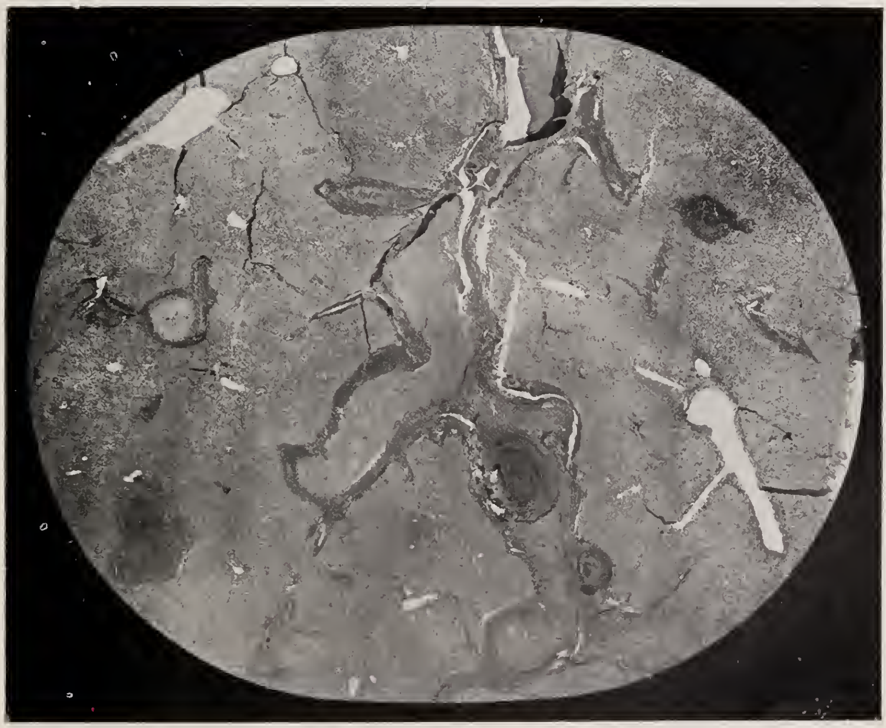

FIG. 11.

Dead schistosomes arrested in the branches of the portal vein. Note the heavy pigmentation. Sheep killed after treatment with tartar emetic. Magn. 14 ×. Spec. No. 8068.

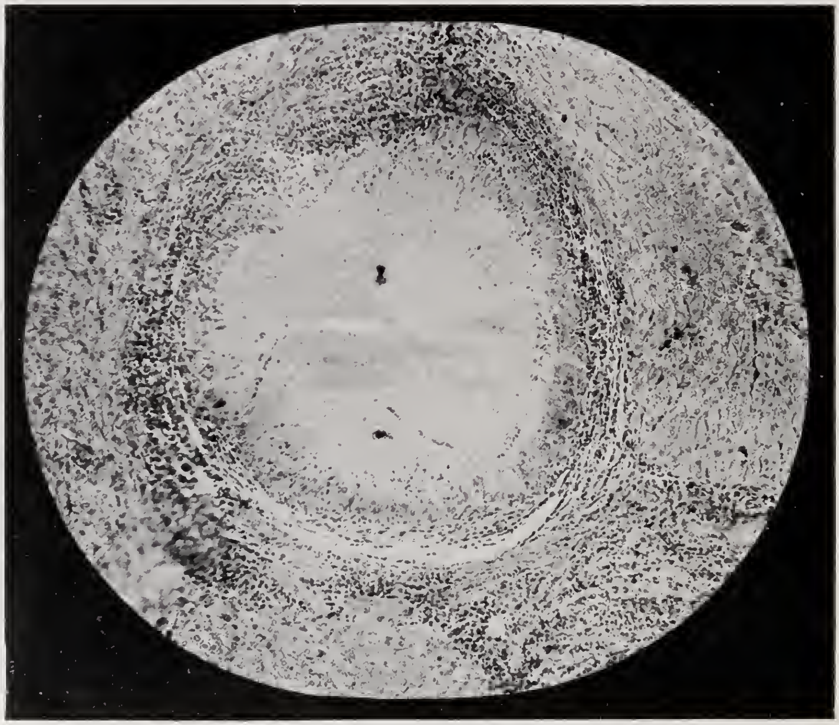

FIG. 12.

Dead male arrested in a branch of the portal vein in the liver. Note cellular accumulation around the parasite. From a sheep treated with Antimony Potassium Tartrate. Magn. 135 ×. Spec. No. 8068.

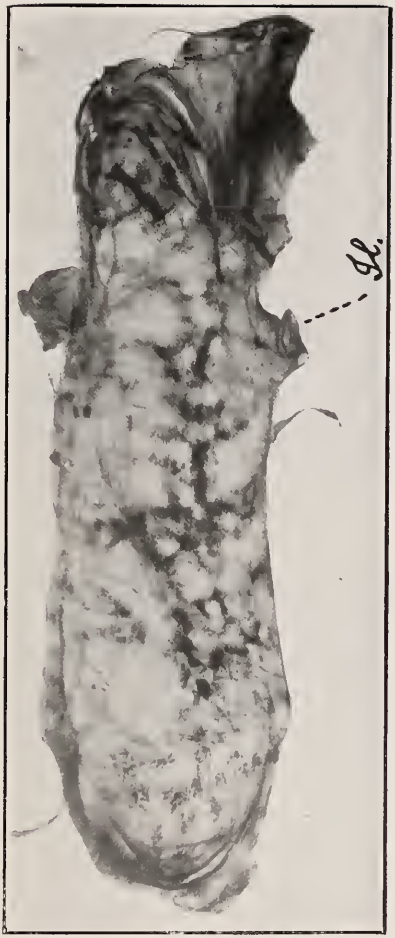

FIG. 13.

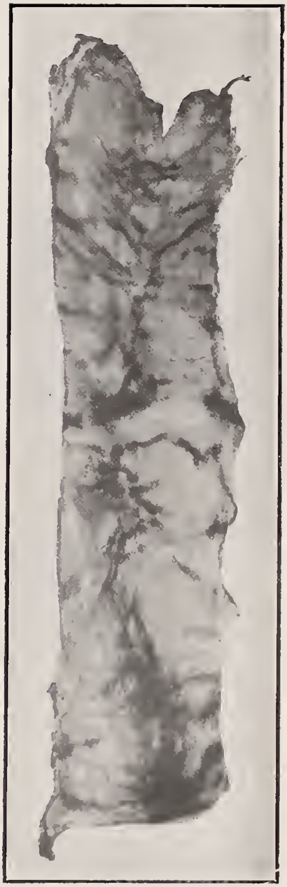

FIG. 14.

FIG. 13.—The distal portion of the caecum showing heavy deposition (dark zones) of eggs in its wall. Sheep killed Magn. $\frac{1}{2}$ ×. Incised along the lesser curvature.

FIG. 14.—A proximal portion of the caecum, same case as Fig. 13.

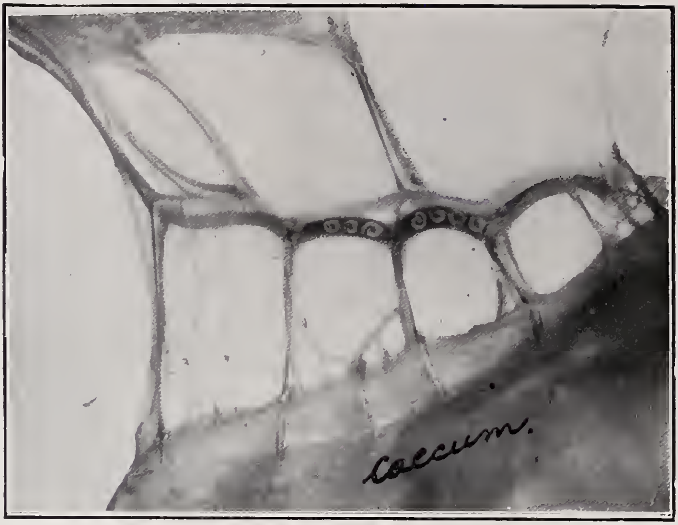

Fig. 15.

Schistosomes in pairs in the veins draining the caecum and the ileum. Sheep killed.

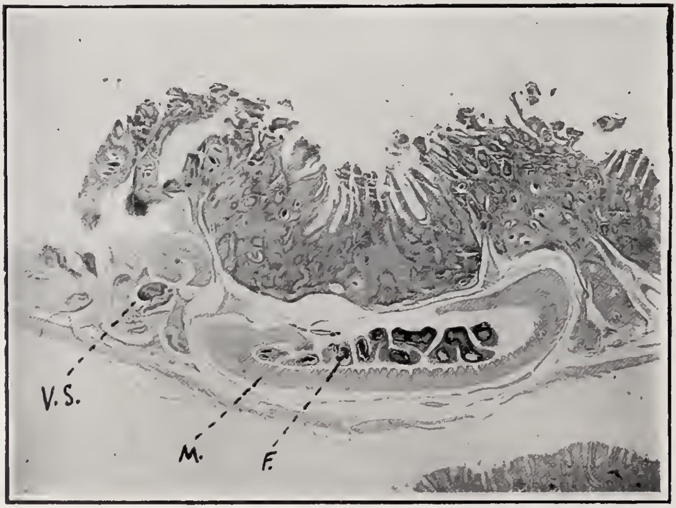

Fig. 16.

Schistosomes in submucosa of small intestine. Pseudo-tubercles in the mucosa propria. Note the ventral sucker (V.S.) of the male. Sheep killed. Magn. 17 ×.

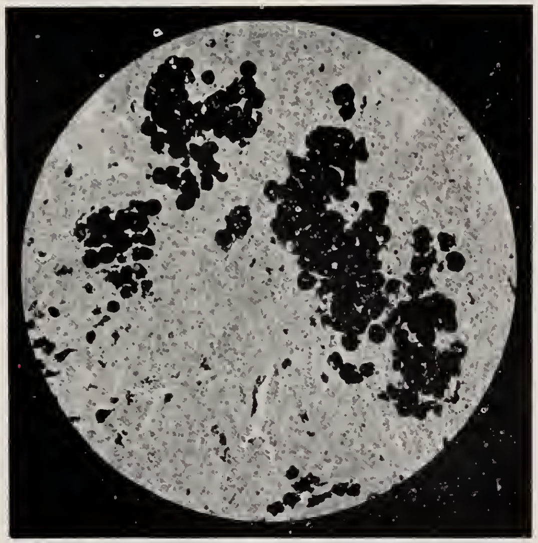

Fig. 17.
Pigmentation of a hepatic lymph gland. Sheep killed. Magn. 135 ×.
Spec. No. 8058.

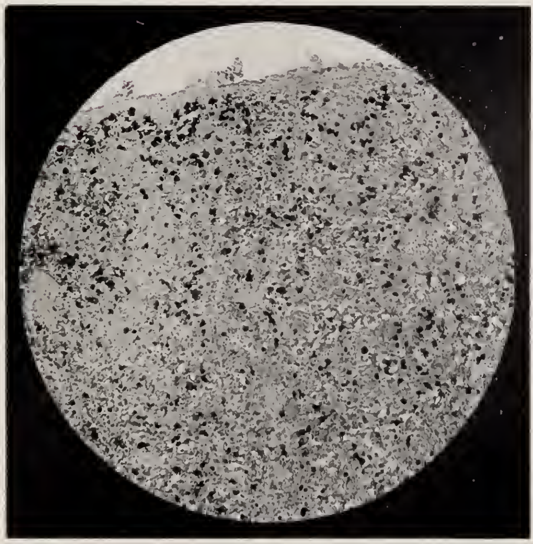

Fig. 17a.
Pigmentation of a hepatic lymph gland. Sheep killed. Magn. 75 ×.

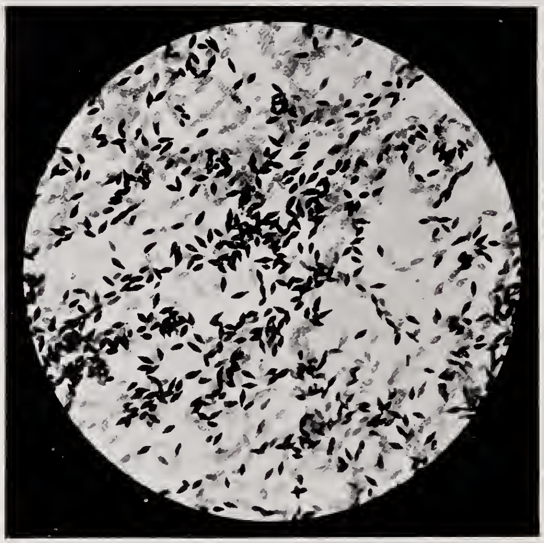

FIG. 18.
Eggs in the mucosa of the small intestine. Sheep killed. Magn. 20 ×.

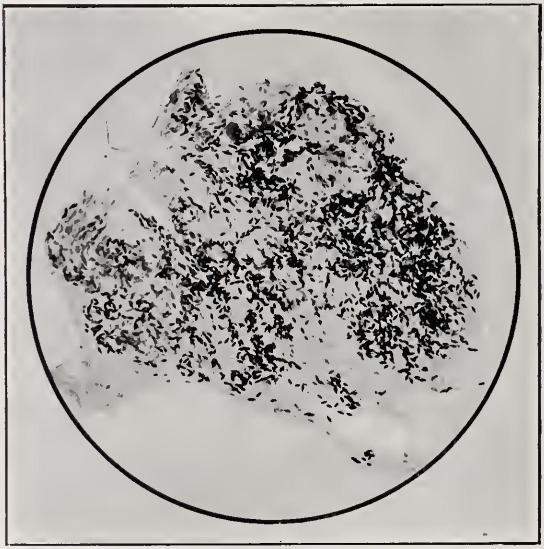

FIG. 19.
Eggs in a piece of mucosa from the caecum. Sheep killed. Magn. 8 ×.

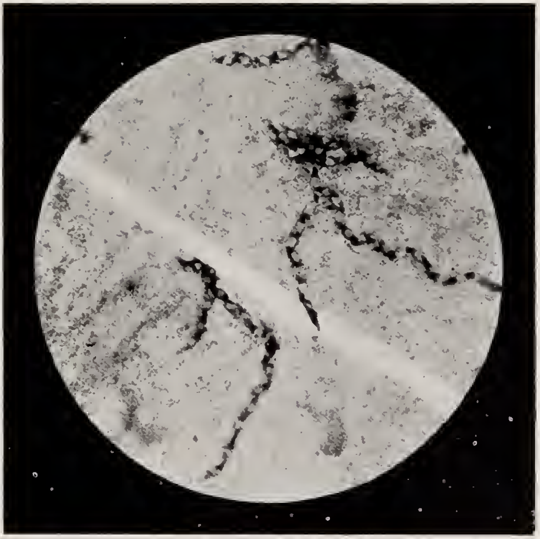

Fig. 20.

Eggs lying in the venules of the small intestines. Guinea-pig, artificially infected with *S. mattheei*. Magn. 35 ×.

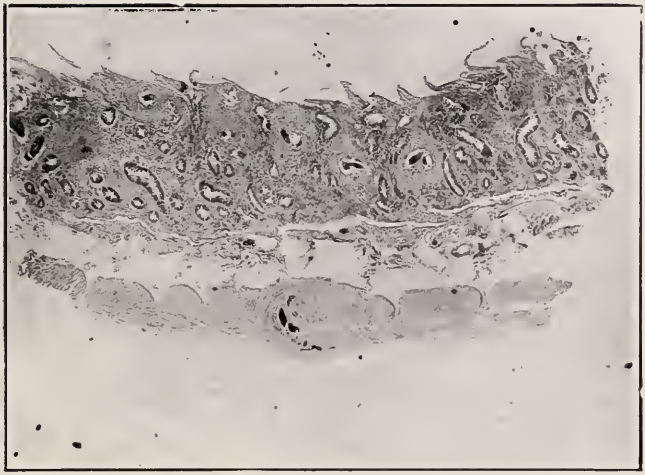

Fig. 21.

Small intestine showing pseudo-tubercles in the mucosa propria, and the muscular layers. Sheep died. Magn. 26 ×.

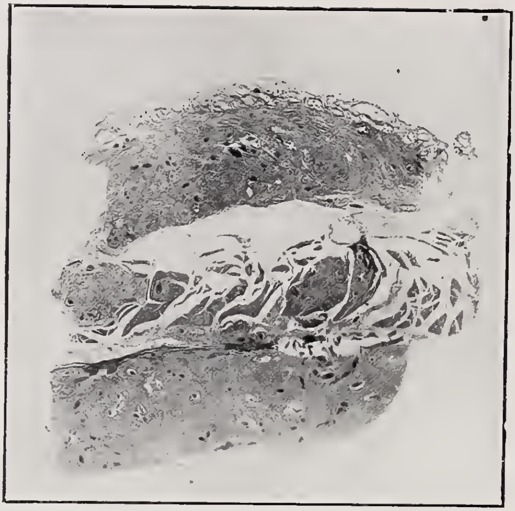

Fig. 22.

Caecum showing pseudo-tubercles in the fibrosed submucosa and muscular layers. Sheep died. Magn. 15 ×.

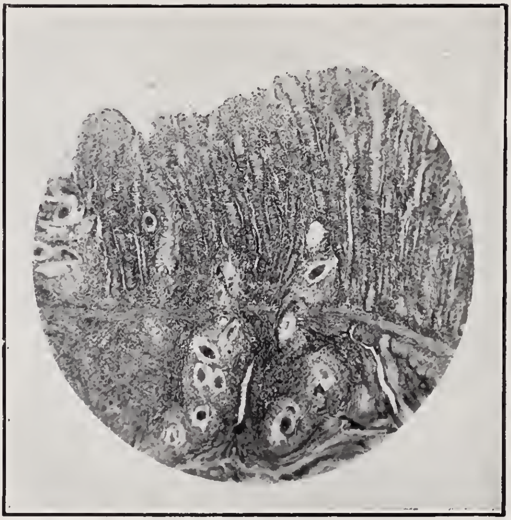

Fig. 23.

Small intestine showing pseudo-tubercles in submucosa and eggs migrating through the muscularis mucosa into the mucosa propria. Sheep killed. Magn. 40 ×. Spec. No. 8323.

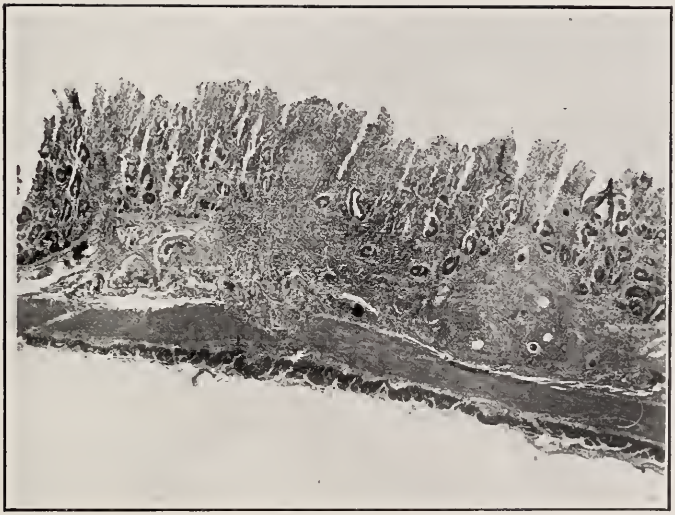

FIG. 24.

Wall of caecum showing pseudo-tubercles in submucosa and mucosa propria. The muscularis mucosa is totally destroyed. Note cellular infiltration of the mucosa propria. Sheep died. Magn. 23 ×. Spec. No. 7887.

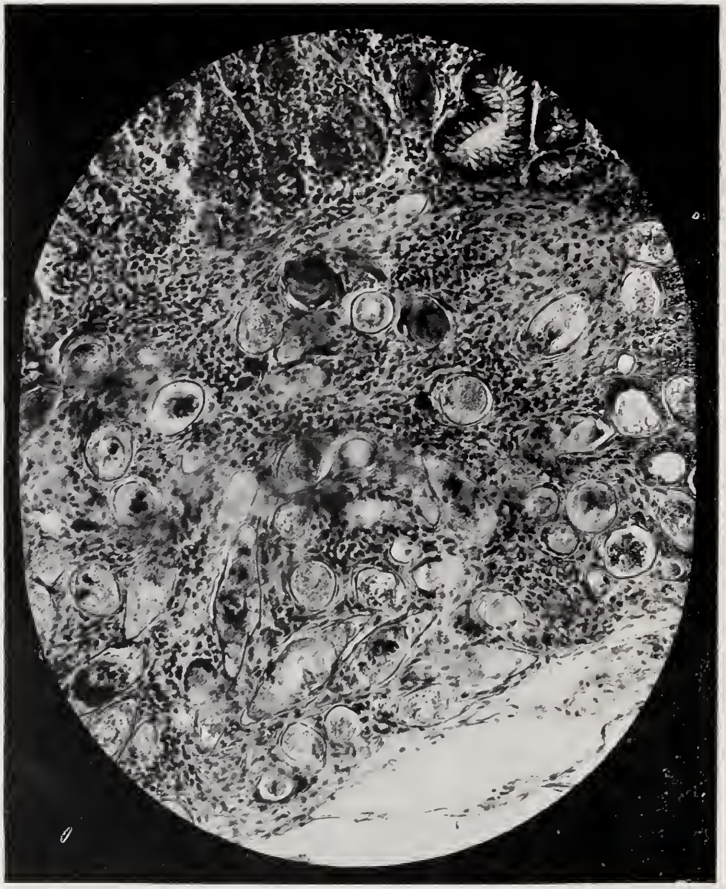

Fig. 25.

Eggs deposited in the submucosa of the caecum. Sheep killed. Magn. 135 ×.
Spec. No. 1862.

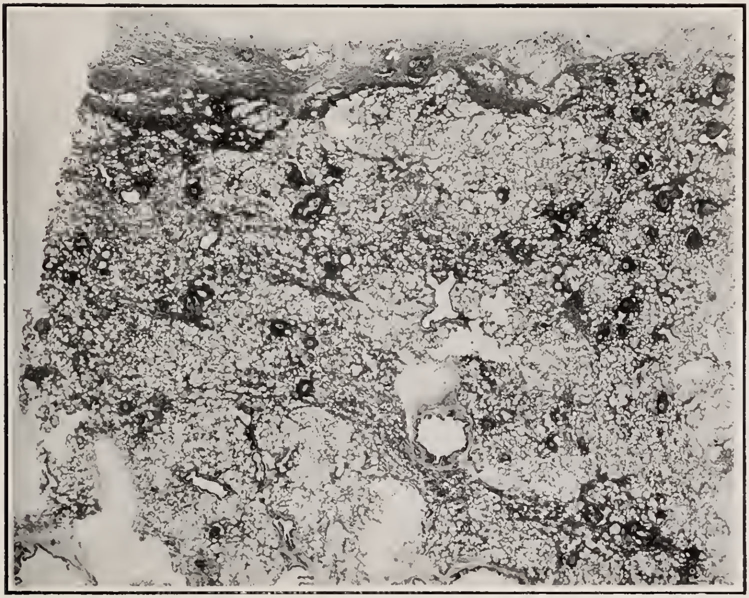

FIG. 26.
Pleura thickened, and numerous pseudo-tubercles. Lung. Sheep killed. Magn. 15 ×. Spec. No. 7783.

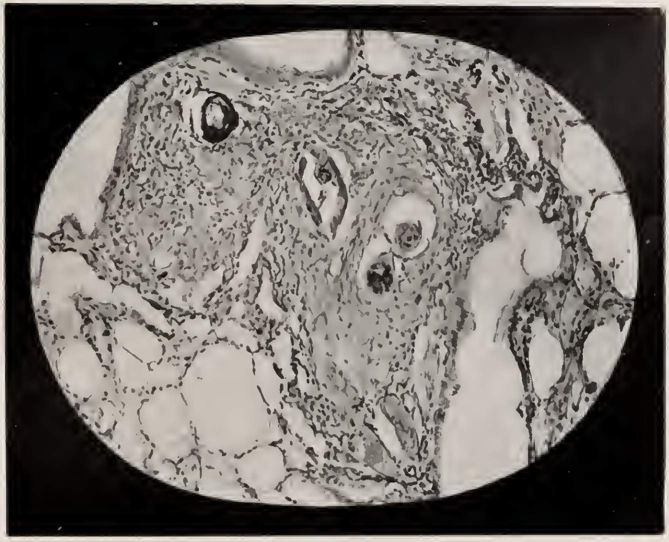

FIG. 27.
Developing pseudo-tubercles. Lung. Sheep killed. Magn. 135 ×. Spec. No. 7783.

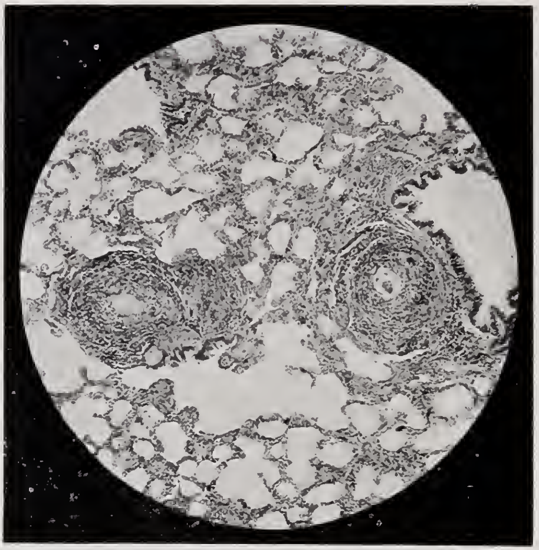

FIG. 28.
Pseudo-tubercles next to a bronchiole. Sheep killed. Magn. 135 ×. Spec. No. 7783.

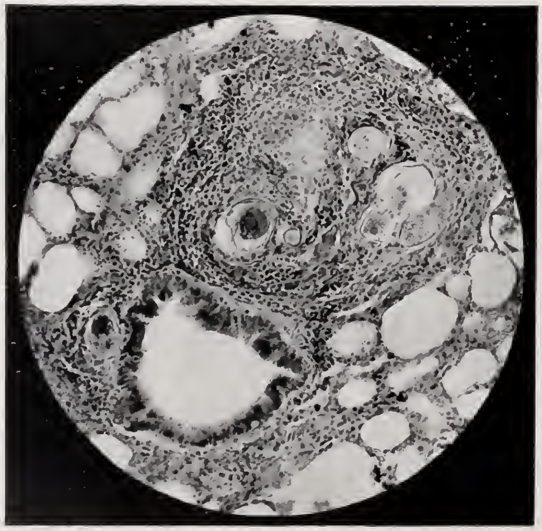

FIG. 29.
Pseudo-tubercles next to bronchiole. Sheep killed. Magn. 75 ×. Spec. No. 7783.

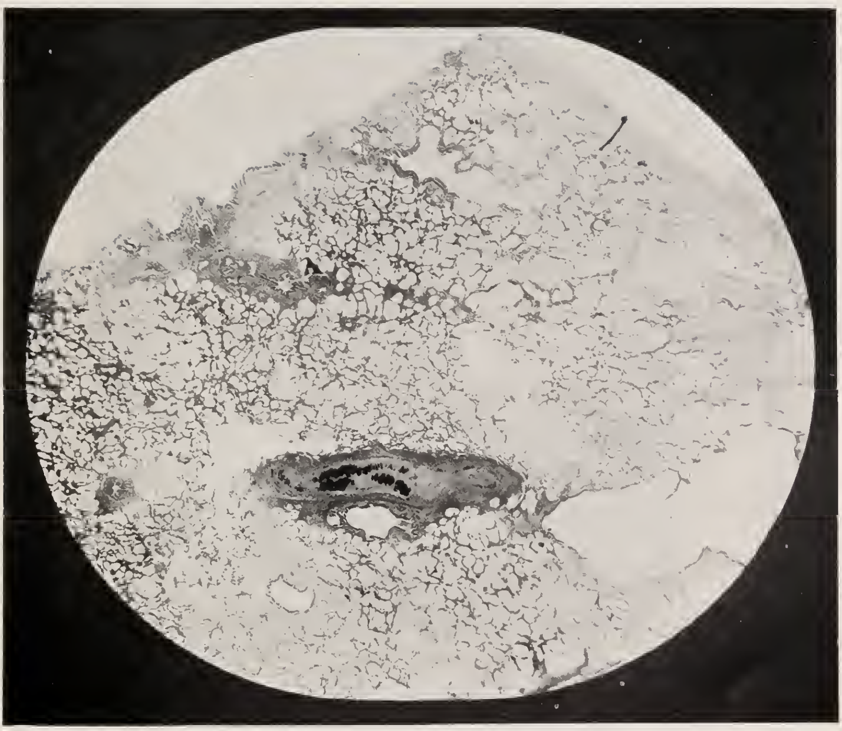

FIG. 30.

A male schistosome arrested in a pulmonary artery. Note the almost complete absence of pseudo-tubercles from this section. Sheep treated with tartar emetic and killed. Magn. 14 ×. Spec. No. 8008.

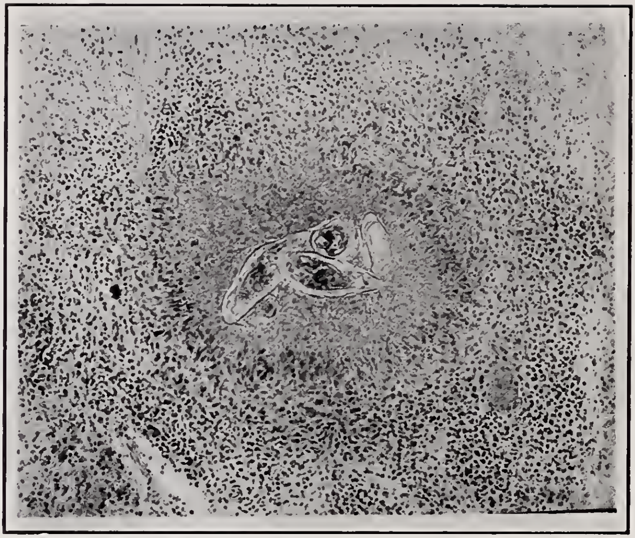

FIG. 31.
Developing pseudo-tubercle in a mesenteric lymphatic gland. Sheep killed. Magn. 120 ×. Spec. No. 8325.

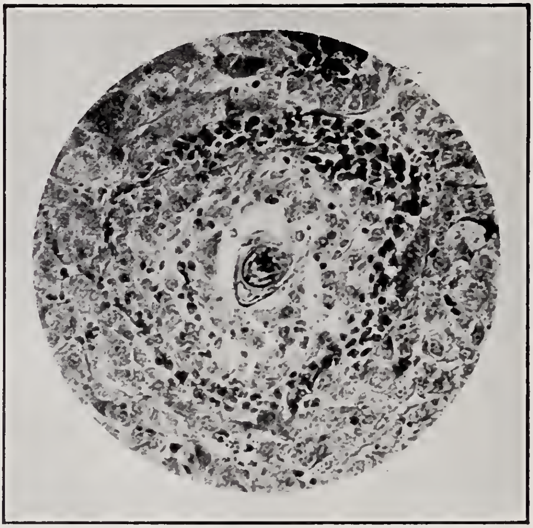

FIG. 32.
Early stage in the development of a pseudo-tubercle. Liver. Sheep killed after experiment via the skin with cercariae of *S. mattheei* from *Physopsis africana* var. *globosa*. Spec. No. 8363.

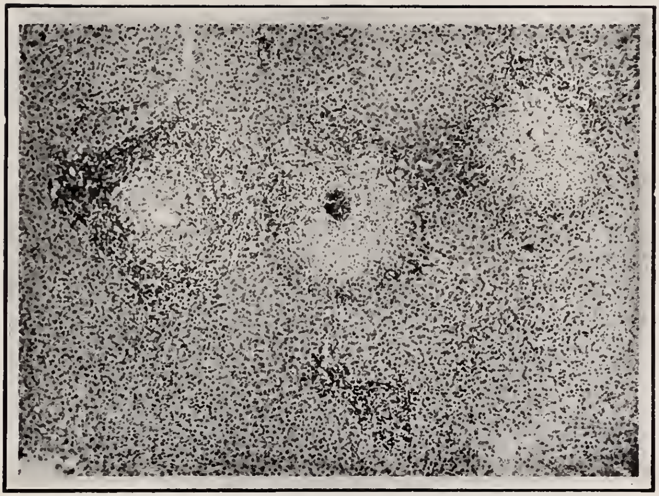

Fig. 33.

Developing pseudo-tubercles. Liver of a guinea-pig experimentally infected with cercariae of *S. mattheei*, obtained from *Physopsis africana* var. *globosa*. Magn. 70 ×. Spec. No. 8624.

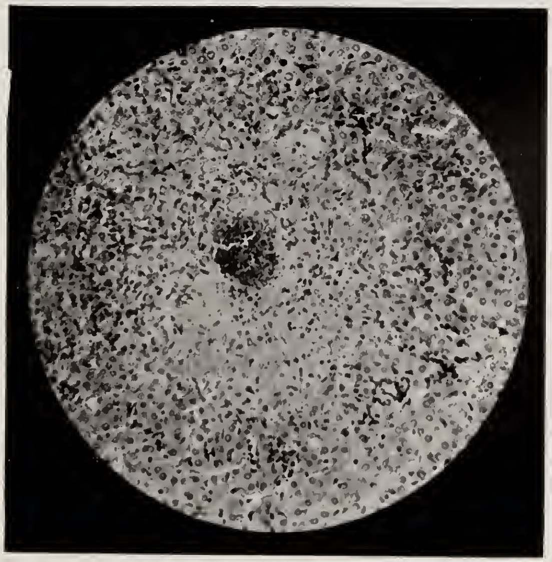

Fig. 34.

Centre pseudo-tubercle from Fig. 33. Magn. 135 ×. Spec. No. 8024.

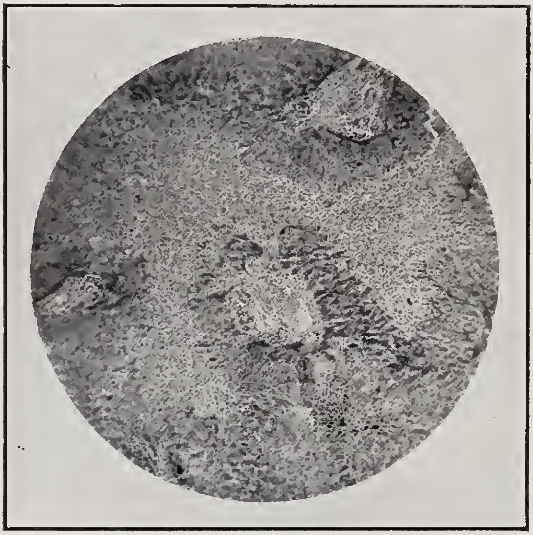

FIG. 35.

Degenerative changes of the liver cells at the centre of the lobule. Cells around the portal tracts not involved to the same extent. Magn. 60 ×.

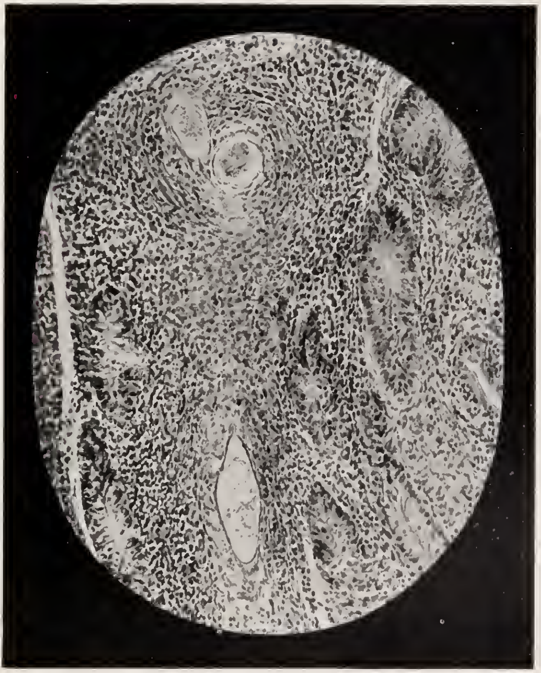

FIG. 36.

Pseudo-tubercle in the mucosa propria and an egg migrating through the tissues. Caecum. Sheep killed. Magn. 135 ×. Spec. No. 8058.

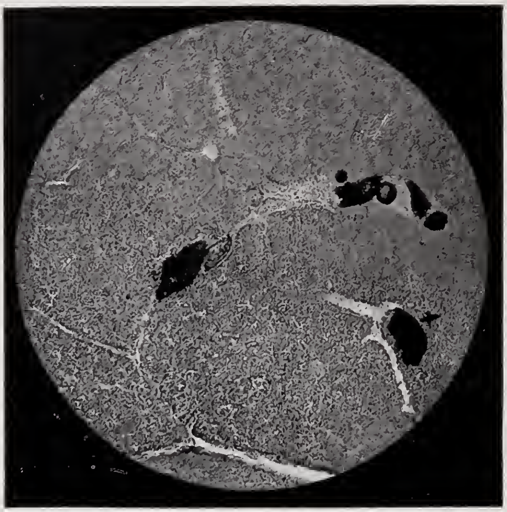

FIG. 37.
Calcified eggs in a pancreas showing a portion degenerated. Sheep killed. Magn. 75 ×.

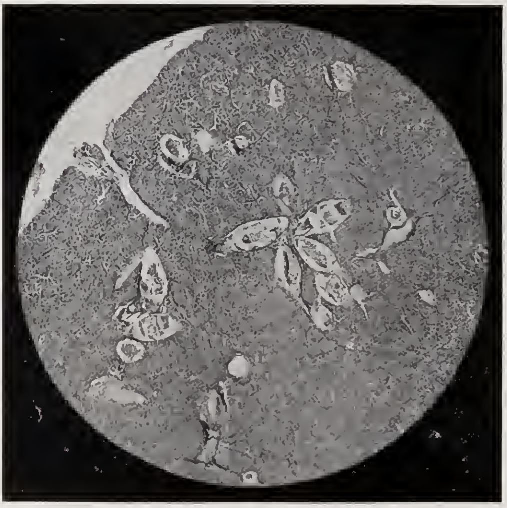

FIG. 38.
Eggs in a lobule of the pancreas. No cellular infiltration is evident. Sheep killed. Magn. 75 ×.

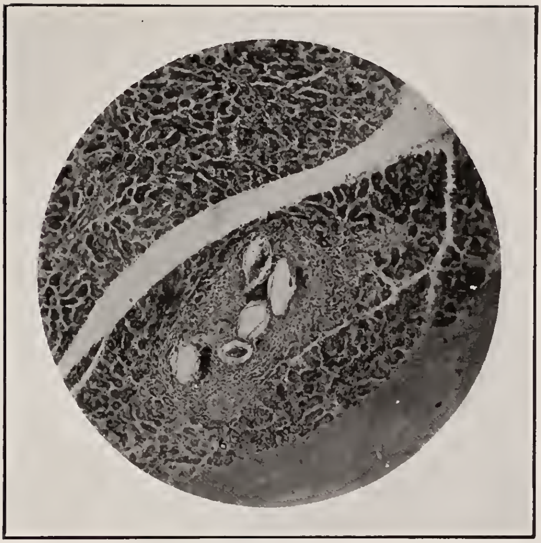

FIG. 39.
Pseudo-tubercle pancreas. Sheep killed. Magn. 60 ×.

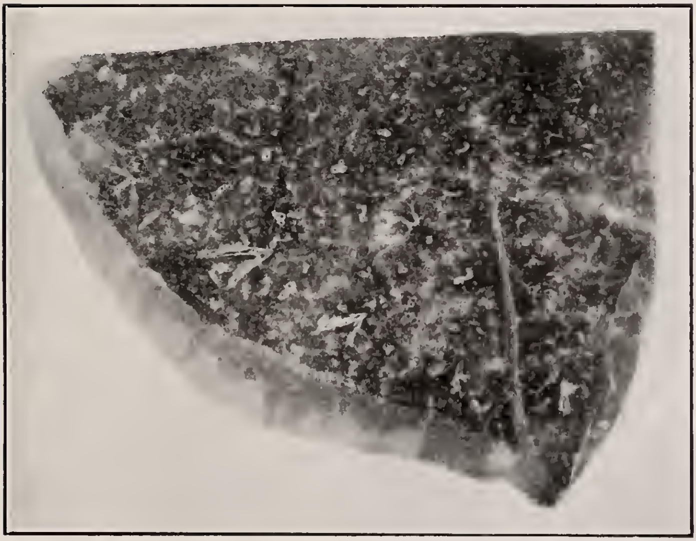

FIG. 40.
Posterior ventral portion of the left lung showing pigmentation. Note the heavy deposition of pigment around the branches of the pulmonary artery. Lobules along the posterior ventral border show hardly any pigmentation. Sheep killed.

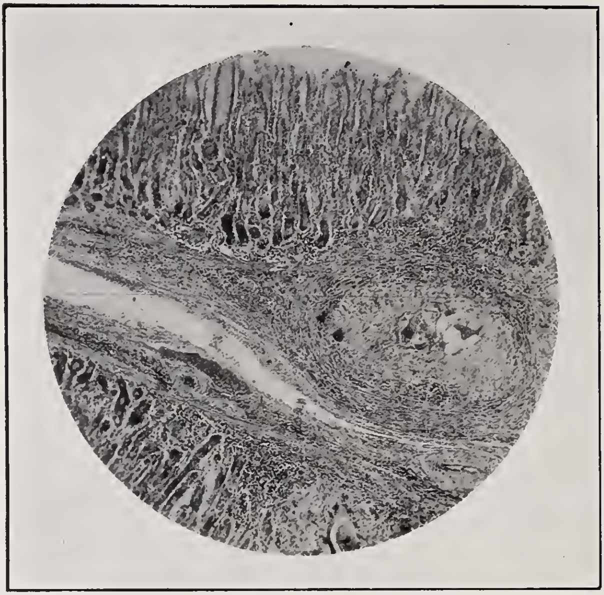

Fig. 41.
Pseudo-tubercle in fold of abomasum. Sheep killed. Magn. 60 ×.

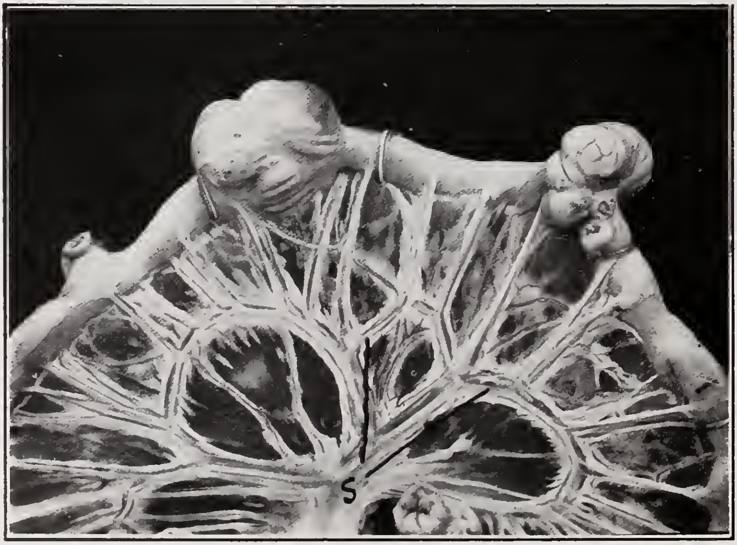

Fig. 42.
Small intestine showing diverticula and calcareous nodules and schistosomes (S) in the veins. Sheep killed. Magn. Natural size.

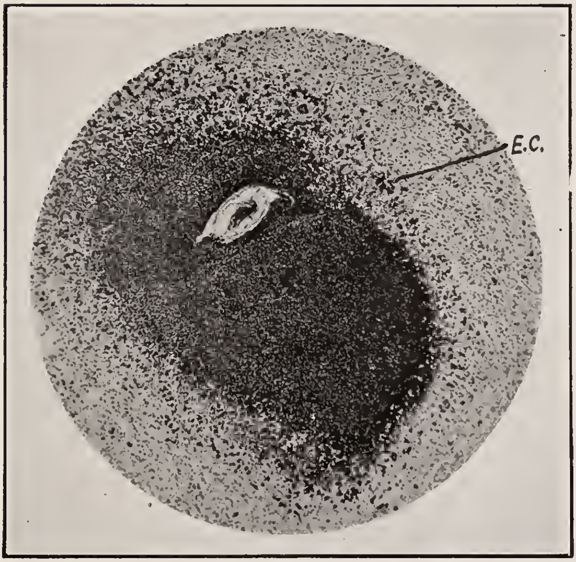

FIG. 43.
Oesophagostomum columbianum nodule, showing the larva, in section, surrounded by leucocytes especially eosinophiles and endothelial cells (E.C.) containing a brownish pigment. Sheep killed. Magn. 70 ×.

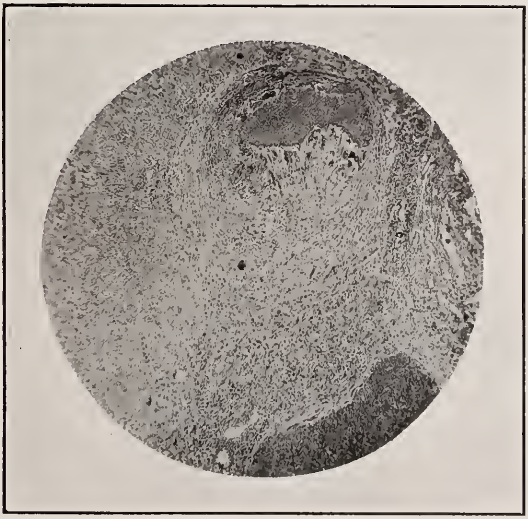

FIG. 44.
Oesophagostomum columbianum nodules, showing fibrous tissue formation stimulated by the larva wandering in the liver. Magn. 35 ×. This field was close to Fig. 43, and indicates a former habitat of the larva. Magn. 35 ×.

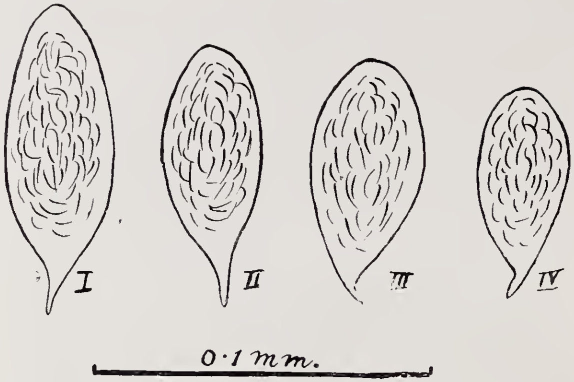

Fig. 45.
Eggs from the uterus of a female which had been subjected to tartar emetic treatment *in vitro.*

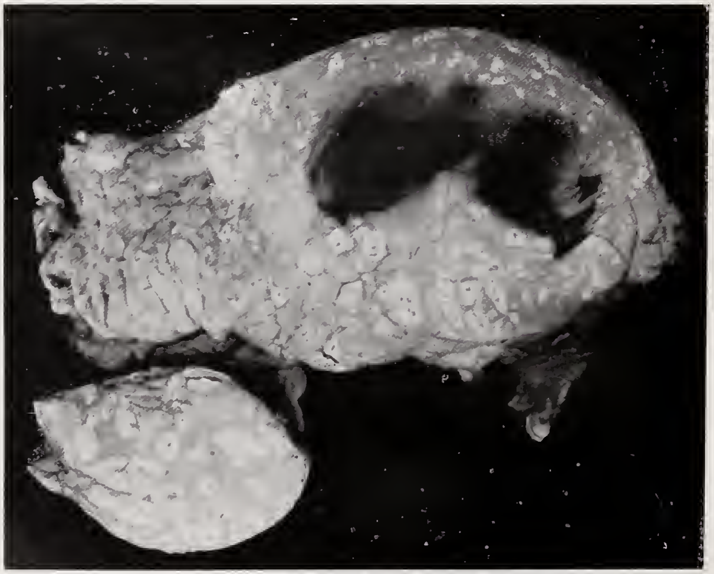

Fig. 46.
Kidney with the perirenal fat showing extensive fat necrosis. Sheep infected with *S. mattheei.*

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

Notes on the Life-Cycle of *Schistosoma mattheei* and Observations on the Control and Eradication of Schistosomiasis in Man and Animals.

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

The life-cycles of the three common human schistosomes have been extensively investigated within the last fifteen years. Miyairi and Suzuki (1913) traced the development of *S. japonicum* in a small amnicolid snail. Leiper and Atkinson (1915) reared the cercaria of *S. japonicum* in the snail *Blanfordia nosophora* (Robson). Leiper returned to Egypt and within a few months proved that *S. haematobium* is transmitted by *Bulinus contortus* (Michaud), *B. dybowskii* Fisher, and *B. innesi* (Pallary), and that *S. mansoni* has *Planorbis boissyi* Potiez and Michaud, as its invertebrate host. It is, perhaps, remarkable that Leiper did not trace the invertebrate host of *S. bovis* (Sonsino) which parasitizes domesticated ruminants in that area.

Other schistosomes which have been recorded from man are: *S. faradjei* Walkiers, 1928, from the Belgian Congo, *S. incognitum* Chandler, 1928, from Bengal in India, and *S. spindalis* var. *africana* Porter, 1926. The first two mentioned were founded on the size and shape of their eggs, which are passed in the faeces. The adults are unknown. The species, *S. spindalis* var. *africana* (syn. *S. spindalis* of Cawston, 1928), was reared in mice, infected with cercariae from *B. tropicus* and *P. pfeifferi*. The snails were infected with miracidia hatched from schistosome eggs, obtained from the urine of two humans in South Africa. The morphology of this species is imperfectly known. From the data available, it is evident that these three species should be regarded as *species inquirendae* until the adults are found.

The size and the shape of the eggs within a species may show considerable variation, as will be evident from the figures (Plate I, Figs. I to VIII and 1 to 7). If the eggs alone are to be considered for the diagnosis of a species, it is evident that this Pretorian was infected with at least four or five different species of schistosomes. There are eggs present reminding one of those of *S. haematobium*, *S. incognitum*, *S. bomfordi*, *S. mattheei*, and *S. spindalis*. It would undoubtedly be most unusual to have one human infected with all these species. The three last mentioned inhabit the radicles of the portal vein in domestic stock and can therefore hardly be expected to prefer the urino-genital tract when localized in man.

The schistosomes recorded from domestic stock are:—

(1) *S. bovis* (Sonsino) Khalil 1924, from cattle and sheep in Africa and Europe (Italy, Sardinia), India, and from cattle in Cochin China.
(2) *S. bomfordi* Montgomery 1906, from cattle in India and Southern France.
(3) *S. spindalis* Montgomery 1906, from cattle, goats, and the water buffaloes in India and cattle in Sumatra.
(4) *S. indicum* Montgomery 1906, from equines and sheep in India.
(5) *S. japonicum* Katsurada 1904, from man and various mammals (horse, ox, sheep, cat, dog, etc.) in the Far East.
(6) *S. turkestanicum* Skrjabin 1913), from cattle in Russian Turkestan.
(7) *S. mattheei* Veglia and Le Roux 1929, from sheep and cattle in the Cape Province.

The invertebrate hosts of some of the schistosomes have been determined and may be briefly considered.

The snails responsible for the perpetuation of *S. haematobium* and *S. mansoni* in Egypt have been considered above. In South Africa the intermediate host of *S. haematobium* was first demonstrated by Becker (1916) to be *Physopsis africana*. His findings have since been verified by Cawston, Faust, and Porter. Porter (1920) also incriminates *Lymnaea natalensis* Krauss, but judging from the prevalence of this snail throughout a large area of the Union and the localization of *S. haematobium* only in areas inhabited by *Physopsis*, it would appear that *L. natalensis* need not be considered as a vector in the spread of schistosomiasis. Porter (1925) names *Physopsis conicum* as a host of *S. haematobium*. In Portugal the host is *Planorbis dufourii* Graells, according to Franca (1922) and Bettencourt and Borges (1922); in Nyasaland it is, according to Dye (quoted by Christopherson, 1923), a species of *Physopsis*, allied to *P. africana* var. *globosa* (Morelet); in Sierra Leone, Blacklock and Thompson (1924) obtained, from *P. africana* var. *globosa*, cercariae which were reared to maturity in animals and proved on examination to be the adults of *S. haematobium;* in Tanganyika Territory the snail, *Physopsis nasutu* v. Martens, is held responsible by Corson, 1927; Ingram (1924) suspects *P. globosa* as the carrier on the Gold Coast; in Cyprus, Leiper (1928) found *B. contortus* prevalent in the endemic area. In Lourenco Marques it is *P. africana* var. *globosa*, according to Cawston (1925).

Schistosoma mansoni is evidently by no means common in South Africa. Porter (1920) records that the South African fresh-water snails, *Physopsis africana*, *Bulinus tropicus* (Krauss), and *Planorbis pfeifferi* Krauss, can serve as intermediate hosts of this blood-fluke. Faust (1920) recognizes the cercariae of *P. mansoni* amongst cercariae from *P. africana* from Natal. In Venezuela, according to Iturbe and Gonzalez (1917), the intestinal schistosomiasis is propagated by *Planorbis guadelupensis* Sowerly. These authors also claim to have artificially infected *Pomacea luteostoma* and *Planorbis cultratus.* In Brazil the snails *P. centimetralis* Ad. Lutz and *P. olivaceus* Spix, are the hosts incriminated by Lutz (1919); in the West Indies it is *P. antiguensis* Gould, according to Jones (1923); while in Dutch Guinea Khalil (1921) found the snail *P. olivaceus* infected; in Madagascar, Haslé (1928) found *Planorbis madagascariensis* infected; Marin (1928) records that Hoffman had experimentally shown *P. guadelupensis* to be the host in Porto Rico.

Schistosoma japonicum (Katsurada) was found by Leiper and Atkinson (1915) to be transmitted by *Blanfordia nosophora* (Robson) (syn. *Oncomelania nosophora; Katayama nosophora; Hypsobia nosophora*). Cort (1919) confirmed the findings of Leiper and Atkinson when he infected rats with cercariae from this snail. In Formosa the host is *B. formosana* Pilsbry and Hirase. In the Yangtze Valley, China, the host was found by Melency and Faust (1923) to be *Hemibia hupensis* (Gredler) (syn. *Melania hupensis; Oncomelania hupensis; Melanis schmackeri*).

Schistosoma spindalis Montgomery, 1906, was found by Liston and Soparkar (1918) to be naturally transmitted by *Planorbis exustus* Deshayes at Bombay. Fairley and Jasudasin (1927) infected water buffaloes experimentally.

Schistosoma spindalis var. *africana* Porter, 1926, is, according to its discoverer, transmitted by *Planorbis pfeifferi* and *Bulinus tropicus.* Bequaert (1928) regards these experiments of Porter as inconclusive.

The life-cycles of all the other animal schistosomes except that of *S. mattheei* are still to be determined. It would surely not be unusual to find that *Bulinus contortus* transmits the so-called *S. bovis* of Egypt, Sardinia, and Sicily.

S. bovis (Sonsino) Khalil is, according to Cawston (1928), transmitted by *Physopsis africana* in Natal. Faust (1921) described, from *P. africana* in Natal, the *Cercaria octadena*, which he believes to be the larval stage of *S. bovis.*

There are cases on record (Cawston, 1921, 1922, 1927, and 1928) where spindle-shaped eggs resembling those of *S. bovis* have been observed in the urine of humans in Natal. Leiper (1923), who had the opportunity of examining some of these eggs observed by Cawston, has doubted this possibility because intermediate forms between these eggs and eggs typical of *S. haematobium* were observed. Brumpt (1922) expresses himself against such a possibility in the statement: " Cette determination est probablement inexacte, cas de dernier ver ne produit que des lésions intestinales chez le boeuf."

Mahfuz (1927) has in Egypt recovered from the urine of a patient suffering from haematuria and painful micturition eggs believed to be those of *S. bovis.* Cawston (1928) writes: " In Natal, however, one sometimes encountered the eggs of other schistosomes associated with those of *S. haematobium*, and adult species of *S. bovis* have been reared from cercariae obtained from *Physopsis*, whilst *S. spindalis* has been successfully reared in the Transvaal and would seem to occur as a rare parasite of man in Natal." Cawston (1928) writes: " I am not aware that bilharziasis has been demonstrated in any animal in South Africa, except in man." In a letter (quoted by Faust, 1926) dated June 29, 1920, Cawston wrote to Faust as follows: "Dr. R. T. Leiper has no hesitation in diagnosing some adult schistosomes from one of my guinea-pigs as *Schistosoma bovis*, which have not previously been found in South Africa; but I have not yet traced the cow (the usual host) that infected the *Physopsis* used in the experiments. To this Faust (1926) remarks: "In fact, the 'cow,' hypothecated by Dr. Cawston, may not be necessarily involved in the *Schistosoma bovis* life-cycle in Natal; man may possibly serve in this capacity." Blacklock (1925), discussing schistosomiasis in Sierra Leone, asks the questions: "Is *S. bovis* present?" and continues: "The position of *S. bovis* appears to be somewhat uncertain, both geographically and morphologically, and also as regards the vertebrate and mollusc hosts. The present vague conception of what *S. bovis* actually is makes it difficult for workers in endemic areas to incriminate snail hosts, unless adult females containing eggs develop in the experimental animals."

In the discussion of Blacklock's (1925) paper, Prof. Leiper refers to the paucity of intestinal infections in Sierra Leone with *S. haematobium* in marked contrast to the findings of Chesterman in the Congo, where *S. haematobium* occurs mostly in the faeces. In Egypt the vesicle infection predominates, and it was because Harley (1864) found only terminal-spined eggs in the urine of his patients at Port Elizabeth that he named *Distomum capense* to differentiate the South African blood-fluke from that recorded from Egypt, where lateral-spined ova were also common.

Nessmann and Trenz have at Gabon in French West Africa found cases of intestinal schistosomiasis without indications of cystic involvement. The eggs passed in the faeces were terminal-spined and were taken for those of *S. haematobium*. No details as to the morphology of the adult parasite are available. The authors do not give figures or measurements of the eggs observed. Reynard and Leger (1922) have in Central Africa seen cases of rectal schistosomiasis without vesicle implication.

It has by no means been proved at autopsy that *S. haematobium* can ever produce intestinal schistosomiasis without involvement of the urinary bladder. Brumpt (1928) records the infection of a hedgehog with *S. haematobium*. He observes that there was no invasion of the urinary bladder. I have not been able to consult the original communication, but from the review of the article in the *Tropical Diseases Bulletin* it is not evident where he obtained the cercariae utilized for infecting the animal. My attempts to rear supposed *S. haematobium* cercariae to the adult stage in sheep, white rats, and guinea-pigs have failed on four occasions. The cercariae used were mammalian schistosome cercariae obtained from *P. africana* var. *globosa*, collected in areas where urinary schistosomiasis is prevalent.

This failure to infect rats with supposed cercariae of *S. haematobium* has been experienced by Ingram (1924) on the Gold Coast, where he tried to infect rats with cercariae from *Physopsis globosa*, Morelet. The *Physopsis* was submitted to miracidium hatched from eggs passed in the urine. He failed to infect *Isidora forskali*, Ehrn. and *Physa waterloti*, Germain.

Here, too, I feel quite satisfied that many of the reports dealing with the rearing of adults of *S. haematobium* in animals other than monkeys are by no means conclusive. It is perhaps not without significance to note that in the guinea-pig, the rats, and the hedgehog, there was no involvement of the urinary bladder, a phenomenon usually observed with *S. bovis* and *S. mattheei*, which both produce terminal-spined eggs. *S. bovis* is morphologically, according to Leiper (1923), closely related to *S. haematobium*. This is borne out by Khalil's description of the female of *S. bovis*. *S. mattheei* is distinguished from *S. bovis* in that the female of the former has the vitelline glands limited to the posterior half of her body, while in *S. bovis* and *S. haematobium* they are limited to the posterior quarter of the body. *S. bovis* may therefore be readily mistaken for *S. haematobium*. Further experiments are necessary to prove that cercariae of *S. haematobium* can be reared to the adult stage in the animals claimed to have been infected. To prove this beyond doubt, it would be imperative that the experimental snails be bred in captivity so as to exclude any possible infection with other mammalian blood-flukes.

I do not doubt the possibility of rearing this human blood-fluke in mammals other than monkeys, but evidence is wanting.

In the cases where I subjected sheep and guinea-pigs to supposed *S. haematobium* cercariae, there is evidence that development did proceed up to a certain point. In Glisson's capsule there were areas showing cellular infiltrations similar to those seen in guinea-pigs and sheep infected with *S. mattheei*. The lungs, too, showed foci consisting of cellular accumulations. In the centres of some of these foci appeared an amorphous mass suggesting dead cercariae or young schistosomes. These lesions were observed in sheep autopsied on the fiftieth day after infection through the skin.

"SCHISTOSOMA MATTHEEI" VEGLIA AND LE ROUX 1929.

Since the invertebrate hosts of the human blood-flukes are known, it was expected that one of them must be responsible for the transmission of *S. matthei*.

Dr. Veglia visited the endemic area, but he arrived there to find the rivers and the vleis (marshes) in flood. His search for snails was futile. Shortly after this he left the Government service and the rest of the investigation was entrusted to me. The endemic area was visited, but once more rain and extreme cold weather rendered the search for live snails of the genus *Physopsis* futile. The presence of a few empty shells proved the presence of *Physopsis africana* var. *globosa*. Leaves of *Nympheae stellata* were examined for specimens, but only specimens of *Lymnaea natalensis* were recovered. They proved non-infected with mammalian schistosome cercariae. The farm was next visited in summer, when numerous specimens of *P. africana* var. *globosa* were collected from the under-surface of the leaves of the blue lotus. The first snail that was examined proved to be infected with a mammalian schistosome cercariae. Fifty specimens were examined, and of these forty-five were infected. Only the larger specimens yielded cercariae. Infected snails could be readily picked out owing to the abnormally pale colour of the invaded liver. Snails were collected for experimental work at Onderstepoort, but very few arrived alive at Pretoria. With the cercariae from these snails I infected three rats, four guinea-pigs, and two young lambs. The animals were born and reared on the station, and were therefore free of schistosome infection. The rats and guinea-pigs were killed at varying intervals between the fiftieth and sixtieth days, and in every case there were recovered schistosomes indistinguishable, except for size, from those parasitizing the sheep in the endemic area. The sheep were passing eggs from the fiftieth day after infection. The one animal was killed for autopsy six months later, and was found heavily infected with the identical species from Humansdorp.

THE CERCARIA OF *SCHISTOSOMA MATTHEEI.*

The study of the morphology of the cercariae from *Physopsis africana* var. *globosa* shows that the integument is armed with spines. In this respect it differs from *Cercaria octadena* Faust 1921, that is regarded as the larval stage of *S. bovis*. The flame cells are arranged as described for *S. haematobium*. In preserved specimens the body measures 160μ to 260μ in length by 65μ to 80μ in diameter. The trunk of the tail varies in length between 180μ to 280μ and has a width of 38μ to 42μ. The *furci* measures from 80μ to 120μ. The dimensions of the anterior suckers were 56μ to 80μ by 40μ to 46μ. The cephalic secretory glands numbered four (two pairs are acidophilic, while the remaining pairs are basophilic). In this respect the cercaria resembles *C. octadena*, with which it may be identical except for the presence of the cuticular spines.

Leiper (1915) considers that animal experiment gave the most satisfactory differentiation between the two species *S. haematobium* and *S. mansoni*. This is undoubtedly a more satisfactory and sure means of differentiating between species than the more laborious method of differentiating morphologically. Blacklock (1925), commenting on the identification of schistosome cercariae, writes: "It would be a great advantage to discover some quicker way of proving a snail to be a carrier of *S. haematobium* infection than the method of animal experiment, and it is possible that some such method may yet be found; owing to the morphological resemblances of *Schistosome* cercariae, it does not seem that diagnosis by this stage is reliable. . . ." "Attraction of miracidia to snails does not appear accurate, nor does the subsequent recovery from the snail of 'typical *S. haematobium* cercariae' until we know what does indeed constitute such."

The development of the miracidium to a cercaria in the digestive gland of the snail is not unlike that described for other schistosomes (the miracidium ⟶ sporocyst ⟶ daughter sporocysts ⟶ cercariae which penetrate the skin or buccal mucosa of the definitive host).

While experimenting with fresh-water snails and the newly hatched miracidia, it was found that the latter are attracted by *P. africana* var. *globosa*, *Lymnaea natalensis*, and a species of *Bulinus*, but not in the least by *Planorbis pfeifferi*. The cercariae would attach to the foot, head, and the antennae of the three first-mentioned snails, but were never observed to penetrate into these parts, even after having been attached for fully quarter of an hour. The normal route of infection seems to be via the respiratory tract.

If a snail is watched in the presence of a few attacking miracidia, it will be observed that those drawn into the pulmonary cavity with the inrushing water are hardly ever returned. By counting the cercariae in a dish of water and then watching the snail, which has been dropped in, it was found that all the miracidia disappeared into the pulmonary cavity. When different snails were simultaneously added, the miracidia seemed to show a slight preference for *Physopsis* and a decided preference for the larger snails. Hitherto it has only been possible to rear the miracidia to the cercarial stage in *Physopsis*, and this was only successful after repeated attempts. Most of the snails would die within a week after subjection to infection. At first the controls could not be kept alive for more than four weeks. *Physopsis* is not easily kept alive under the conditions here. By continually adding leaves of the blue lotus they seem to do much better.

The other snails (*Lymnaea natalensis*, *Bulinus* sp., and *Planorbis pfeifferi*) bred well in captivity.

BIONOMICS OF *P. AFRICANA* VAR. *GLOBOSA* IN THE ENDEMIC AREA.

Blacklock and Thompson (1924) remark that *Physopsis c.f. globosa* Morelet was found " in waters which lay or ran slowly on a muddy bottom, where weeds or grass grew in the water, and under high or low shade." At Humansdorp this species was found in localities where the river had overflowed and had formed pools off its main course or where the main course had been dammed up. I can confirm the observations of the two workers, quoted above, except that as regards shade the opposite was the case with the carrier of *S. mattheei*. Local climatic conditions may account for this variation. Humansdorp is situated about fifty miles south-west of Port Elizabeth and the average temperature is undoubtedly below that of Sierra Leone. At Humansdorp the snails were most numerous on the under-surface of the leaves of the blue lotus growing in direct sunlight and where the bottom of the pool was covered with decomposing vegetable matter mixed with mud. The snails seem to favour those pools where the depth of the water varies between three and four feet.

CAN *SCHISTOSOMA MATTHEEI* INFECT MAN?

Judging from the fact that the pool responsible for the infection of the sheep is used by man and beast alike, it is only natural to assume that if man could be infected, the human beings on the farm should have been. Both urine and faeces were examined with negative results. The infected pool is about two miles long, but only about twenty yards wide and, except at the one end, very deep.

On my second visit there I suggested bathing in that portion where there were no snails, but the young fellows assured me that my skin would " burn " for hours if I did. This, they informed me, was especially the case towards the end of summer. I next tried to get them to have all the lotus leaves removed so that I could test the effect of copper sulphate on the snails. They could not be persuaded to enter the water. Two young natives (ages about 16 and 19) were engaged to clear the portion measuring about twenty yards by twenty-five yards. After having been at it for two hours, they were continually scratching the more tender parts of their bodies. Needless to say, they never cleared the pool. Their faeces and urine were examined the next day, two months and three months later, but always with negative results. Although these findings are perhaps by no means conclusive, they seem to disprove the possibility of man normally functioning as a definitive host for *S. mattheei*. At first I was inclined to suspect this parasite as responsible for those cases of intestinal schistosomiasis, without cystic implication, associated with terminal-spined eggs as recorded from Central Africa, but my observations in Humansdorp seem to disprove this possibility.

It is undoubtedly noteworthy that, although vesicular schistosomiasis has been known in Port Elizabeth for over half a century [Harley (1864) records the first cases], the infection has never spread to Humansdorp. Dr. Wentzel, a retired medical practitioner, informed me that he had never met with a single case locally.

Schistosomiasis is by no means as widespread in the Cape Province as suggested by the chart (Fig. 584) published in Byam and Archibald. That chart is a misstatement of facts. Judging from the public interest shown in the schistosomiasis question, the disease is probably not as widespread as some would have us believe. The disease seems localized to certain well-defined areas.

THE ACTION OF TARTAR EMETIC, EMETINE HYDROCHLORIDE AND SB. 212 ON "SCHISTOSOMA MATTHEEI" IN THE MERINO.

This is a preliminary communication on the results obtained with certain chemicals against a schistosome infesting sheep and cattle in the Cape Province.

Literature dealing with the treatment of schistosomiasis in sheep is apparently non-existent. Fairley (1924 and 1926) reports on his results with tartar emetic and emetine hydrochloride against *Schistosoma spindalis*, Montgomery 1906, in goats. These goats were experimentally infected. He records that tartar emetic, administered intravenously in doses of 3.9 to 5.5 mg. per kg. body weight and continued daily for sixteen to twenty-six days, is capable of curing *S. spindalis* in goats. Emetine hydrochloride was found to be effective when administered in doses of 0.7 to 1 mg. per kilogram body weight, and continued daily for ten to fifteen days. He observes that this drug proved to be quite as specific as tartar emetic, but is more toxic to the host.

The literature treating with the curative effects of tartar emetic and emetine hydrochloride in human cases is voluminous. Each drug has its champions. Fairley (1926), in a concise but comprehensive summary, enumerates the findings recorded by various workers.

In the discussion which followed Fairley's paper (1926), several of the pioneers in the campaign against schistosomiasis stated their views. Christopherson, a great believer in tartar emetic, expressed the opinion that emetine hydrochloride is neither as efficacious nor as safe as tartar emetic, and that schistosomes may become "fast" or "tolerant" to either of these drugs. He next remarked that Fairley did not mention the direct action of tartar emetic and emetine on the eggs in the tissues, though he (Fairley) indicates that there is an indirect action through the females. Christopherson concluded by stating that tartar emetic acts directly on the parasite and not indirectly through the tissues of the host, as Fairley is inclined to conclude from his experiments with tartar emetic and emetine hydrochloride on schistosome cercariae *in vitro*.

Day followed and observed that, weight for weight, boys required larger doses than adults. The next speaker was Hodson, who wanted to know whether eosinophilia would not be a more suitable clinical test than the complement fixation reaction, advocated by Fairley. Low referred to the fact that Fairley found, in treated animals, males with the females absent or reduced in numbers, and concluded that some of the clinically cured human cases might still harbour males. He concluded by pointing out the importance of antimony in Tropical Medicine, where its compounds are to-day utilized for curing *schistosomiasis*, *trypanosomiasis*, *kala-azar*, and *ulcerating granuloma*.

Clayton Lane observed that Fairley's findings indicated that, as with quinine and the malaria parasites, it was not the drug which killed the parasites, but a derivative of it formed in the body of the host. Wenyon was not prepared to accept Fairley's conclusions as regards the indirect action of the drugs, and rightly suggested that the serum of an animal which had been treated should be compared with normal serum as regards its lethal properties on cercariae *in vitro*. Manson-Bahr concluded the discussion by stating that he had found Von Heyden "471" valuable in kala-azar, but quite useless against schistosomiasis.

That the antimony compounds do act indirectly on the schistosomes cannot be wholly denied and is evidently not without a parallel in helminthology. The action of carbon tetrachloride on the members of the genus *Fasciola* seems to be indirect. It is now generally accepted that this drug has no action on the immature flukes located outside the lumen of the bile-ducts. While testing carbon tetrachloride against the common liver-fluke of sheep in the eastern Transvaal in 1926, I observed that the flukes, collected from the gall-bladder and the bile-ducts twenty hours after the administration of the drug, appeared with the posterior portions of their bodies necrosed. The tissues in the immediate neighbourhood of the suckers appeared apparently normal (Fig. I). It was undoubtedly the degenerative changes in the cuticle and the posterior portion of the body that rendered the cuticular spines functionless and led to the expulsion of the parasites. Liver-flukes were then collected from sheep at the municipal abattoir at Ermelo and dropped into pure carbon tetrachloride and others into a mixture of this chemical and raw linseed-oil. They were kept in these fluids for twenty hours without showing the changes observed *in vivo*. This, together with the fact that immature flukes escaped, seems to indicate that carbon tetrachloride acts indirectly on *Fasciola*, and that the compound responsible for the death of the parasite is formed after the drug reaches the bile-ducts.

With an increased dose (the dose required to kill liver-flukes is 1 c.c. of carbon tetrachloride) the degenerative changes were more marked after twenty hours than with the minimum dose.

Some authors have attributed the fatality following the use of carbon tetrachloride in infected animals to toxins liberated by the decomposing flukes. Experiments at Onderstepoort have shown that young cattle in good condition and free of fluke infection are very susceptible to carbon tetrachloride and could be poisoned by a dose (10 c.c.) which produced no visible ill-effects in old Merino ewes which were badly infected with flukes.

Minot (1927) has suggested that the lack of calcium in an animal's body may increase the toxicity of the drug. Certain workers on the mineral requirements of the animal body claim to have proved that the presence of magnesium has an influence on the calcium metabolism. This may explain the apparent decrease in the toxicity of carbon tetrachloride when magnesium sulphate is administered simultaneously with the drug.

The failure of male-fern preparations to kill the immature flukes which are still located in the liver tissue outside the bile-ducts suggests that the active principle of these drugs acts indirectly.

THE INFLUENCE OF TARTAR EMETIC ON THE SCHISTOSOMES *IN VIVO*.

Two sheep, in poor condition and weighing 39 and 43 pounds respectively, received intrajugularly 36 grains of tartar emetic. Each animal received 4 c.c. of a 6 per cent. solution every other day. When the day for injection fell on a Sunday, the dose was administered on the Monday. The first dose was received on the 23rd April, 1928, and the last one on the 9th May, 1928. One animal was killed on the 28th May, 1928, for autopsy and showed a few very small schistosomes, in pairs and singly, in the smaller veins of the mesentery. A few were also collected from the hepatic ramifications of the portal vein, the right ventricle, and the pulmonary arteries and their branches.

Emboli containing dead parasites were numerous in the ramifications of the portal vein in a markedly cirrhotic and pigmented liver. Emboli, caused by the arrest of dead schistosomes in the smaller branches of the pulmonary arteries, were frequent in both lungs. Males and females were present in equal numbers. The females, being dark in colour and thinner than the males, had to be specially looked for.

The effect of tartar emetic in influencing the habitat of this schistosome has been stressed in another publication (Le Roux, 1929).

SOMATIC CHANGES OBSERVED IN SCHISTOSOMES SUBJECTED TO THE ACTION OF TARTAR EMETIC *IN VIVO*.

I have been unable to trace any communication recording somatic changes in schistosomes subjected to the lethal action of either tartar emetic, emetine hydrochloride, emetine periodide, Sb. 212, carbon tetrachloride, or any other drugs. The presence of deformed eggs *in utero* has been recorded from females that had been subjected to drugs injurious to schistosomes.

The microscopic examination of specimens, recovered from the treated sheep, showed marked somatic changes from the normal. The parasites were much shrunken both in length and in breadth. The length was reduced to approximately one-fourth of the normal. The intestinal caeca, in the male, presented a most tortuous course, indicating that shrinkage in the length of the gut was not proportional to that manifested by the body. This latter phenomenon was even more pronounced in the dead specimens arrested in the liver and in the lungs.

The testes were reduced in size and their contents were more translucent than normally.

The muscular layers of the body were reduced in depth.

The microscopical examination of the females revealed conspicuous changes in the genital organs. The uterus contained either no eggs, or only a few. The eggs present were almost invariably reduced in size and with the spine abnormally shaped (Figs. 37, 38, 39, and 40). The ovary was decreased in size, and its contents abnormally translucent. The common intestinal caecum, empty and tortuous, was surrounded by vitelline glands which were no longer of that characteristic dark colour as seen in the normal parasite.

THE ACTION OF TARTAR EMETIC ON THE OVA *IN VIVO.*

Christopherson's view has been stated. It has its supporters and its opponents. Neither group has proved its case beyond dispute. Much more experimental work is to be undertaken to prove or disprove either the one or the other view held. Before their hypothesis can be accepted as proved Christopherson and his supporters will have to ascertain the time required by the egg to reach the lumen of the organ in the wall of which it was deposited.

Maciel (1926) records that the eggs of *S. mansoni* are passed out of the body normally within twenty-four hours of being laid. He further observes that a low barometric pressure increases the egg output. He attributes this increase to influence on the worm. There is no proof that the female is induced to lay more eggs. The passage of the ova through the tissues is more probably affected.

The faeces of the autopsied sheep yielded live miracidia up to the time of killing. From the fifteenth day after treatment there was a most marked decrease in the amount of bloodstained mucus passed. When the animal was autopsied there were still eggs, containing live miracidia, present in the mucosal scrapings. There was a most significant decrease in the number of eggs present in the faeces. The other sheep passed no live eggs after the fifth week.

EMETINE HYDROCHLORIDE AND SB. 212.

Emetine hydrochloride was administered as recommended for goats by Fairley (1926). The results on the two animals used were most disappointing. The presence of schistosomes, slightly reduced in size, in the lungs of the animal killed a month later proves that it had some effect. The second sheep was killed four months later and normal schistosomes were recovered.

The price of this drug will limit its use in the control of schistosomiasis in domestic stock.

Sb. 212 was tried in double the dose recommended by Khalil for humans. The results were even more disappointing than with emetine hydrochloride. No other drugs have been tried to date.

The two last-mentioned drugs can be administered subcutaneously or intramuscularly without ill-effects, as has been proved by experiments on normally healthy sheep.

Excessive doses of emetine hydrochloride caused a marked hyperaemia of the membranes of the mouth, pharynx, and larynx. On continuing the dose, the hyperaemia was followed by oedema and ultimately ulcerations and necroses of the mucosa of the parts mentioned above. These lesions were accompanied by similar lesions of the mucosa of the oesophagus, rumen, reticulum omasum, abomasum, and the small intestine. The two last mentioned were badly affected. Degenerative changes were present in the liver.

THE QUANTITY OF TARTAR EMETIC REQUIRED TO EFFECT A CURE IN INDIVIDUAL CASES.

It has been repeatedly stated that, weight for weight, boys require more than adults. This may be due to (i) the absence in boys of a substance which interacts with the drug and forms a compound or compounds lethal to schistosomes; that the drug may indirectly affect the parasites seems quite probable; and (ii) the reduced concentration of the drug in the circulation.

It seems quite logical to expect that the minimum amount required to kill the parasites in an adult will also be the minimum lethal dose for those parasitizing children.

In Byam and Archibald (1923) the treatment recommended for humans consists of two courses with an interval of seven days between them. Each course consists of a series of five intravenous injections, one being given every second day. According to them, adults will have received 18.5 grains and boys about 13 grains of tartar emetic at the end of the second course. Khalil (1926) gives three injections per week for three weeks. The tartar emetic is given in a 6 per cent. solution (1 c.c. of this solution contains about 1 grain of the drug). His first dose is 0.5 c.c. This is increased to 1 and 1.5 c.c. for the second and third doses respectively. For the rest of the course the dose is 2 c.c.

Khalil's system is much preferable to that recommended in Byam and Archibald (1923). The interval of seven days undoubtedly favours the survival of parasites.

Khalil found sodium antimony tartrate as efficient as tartar emetic. This salt is more expensive and much less stable than the latter. He also tried Sb. 212, and recommends it for children and fat individuals where intravenous injections are not advisable, but where intramuscular injections are more practical. This drug has been used on sheep in the field and had, according to the farmer, beneficial effects. My experiments at this Institute did not verify this. It must be stated, though, that the worse affected animals were bought for experimentation.

The Sb. 212 used had been in store for over two years, and several of the bottles showed that the salt had changed to a partly insoluble mixture. On dissolving it in water a reddish deposit was precipitated.

THE TECHNIQUE EMPLOYED FOR INTRAVENOUS ADMINISTRATION OF TARTAR EMETIC IN SHEEP.

The sheep to be treated are removed from the general flock and the wool clipped off the right side of the neck. The wool is closely clipped over the course of the jugular vein. This is best accomplished with curved scissors.

The following morning the first injections are given and repeated every other day. The first injection of 2 c.c. of a 6 per cent. solution is followed by a second of 3 c.c. and a third of 4 c.c., which is continued until the tenth injection has been given. The 6 per cent. tartar emetic should be freshly prepared every time and sterilized by boiling for a few minutes. By using freshly prepared solutions hardly any oedema develops at the site of inoculation, and the ten injections can be administered on the same side. Where several sheep are to be inoculated, it is advisable to use a 20 c.c. syringe and to have the solution ready in bottles holding 100 c.c. each. A few spare sterilized syringes and a needle for every five sheep should be at hand.

For controlling the animal on its side on the ground, two assistants are required. One holds the animal's feet and the other presses on the jugular vein at the base of the neck and holds the syringe while the needle is inserted. The animal's head and neck are controlled by the right knee and the left foot of the operator kneeling at its side. The right knee is placed behind the animal's neck and the heel of the left foot pushed against the jaw holds the head extended in a straight line with the neck. The needle is inserted into the distended vein, and as soon as blood flows from it the syringe is connected, the assistant releases his hold on the vein, the required amount of solution is injected, and the sheep is released to wander off at ease.

With willing assistants it is possible to do at least eighty or more sheep an hour.

When emetine hydrochloride is used intramuscularly, it is best administered into the muscles of the thigh. A much thinner needle is now used. The selected limb should be firmly held during the operation.

These operations should, for preference, never be attempted in the kraal or shed as is commonly met with in South Africa. When the operation is performed in a kraal or near it, there is a great risk of inoculating the animals with virulent gas-gangrene producing organisms.

Prior to the operation, the site of inoculation is rubbed with cotton wool soaked in 5 per cent. carbolic acid in soft water.

OBSERVATIONS ON THE CONTROL OF SCHISTOSOMIASIS IN MAN AND ANIMAL.

Within the last fifty years the life-cycles of at least twenty trematodes parasitizing man and his domestic mammals and birds have been determined. The life-cycles of seventeen species have been determined since 1915.

Various authors, bearing in mind these findings, have hypothecated measures for the control and the eradication of trematode diseases. Some of the measures suggested have evidently never been put to the test; at least, there are no records of their successful application. Others seem rather impracticable for general application.

That all mammalian schistosomes require molluscs for their successful propagation in a given locality can no longer be disputed. It is, therefore, evident that the vulnerable points in their life-cycles, to be aimed at, are:—

(1) *The adult parasite*, which may be successfully exterminated by irrigating the veins of the infected vertebrate host with tartar emetic or any other drug lethal to schistosomes.
(2) *The miracidia*, which should be prevented from reaching its hosts by preventing infected urinary and faecal matter from gaining access to the snail-infested waters.
(3) *The intermediate host*, which can be eradicated by the administration of poisonous substances to the infested waters, by drying, or by other means.
(4) *The cercariae*, which should be prevented from reaching the definitive host.

(1) THE ERADICATION OF THE ADULT SCHISTOSOMES FROM THE DEFINITIVE HOST.

Although MacDonagh had good results with tartar emetic in a few cases during the Boer war, he only published his findings, to which he seemed to attach little importance, in 1917. To Christopherson (1917), from Khartoum, is due the credit of bringing the use of tartar emetic to the notice of a world clamouring for a specific cure against that debilitating disease *Schistosomiasis* (Vel *Bilharziasis*).

Hutchison (1913) was evidently the first to note the efficacy of emetine hydrochloride against the Eastern schistosome (*S. japonicum*). It has since been proved lethal to other schistosomes. Most practitioners seem to prefer using tartar emetic to emetine hydrochloride, which is said to be somewhat toxic to the host.

Organic compounds of antimony have been experimented with and good results are claimed for Bayer *Sb.* 212 (Khalil, 1926), and *Antimosan* [Orenstein (1928), Specht (1926), and others]. Cawston (1929) refers to Orenstein's findings, and expresses the view that antimosan is likely to prove as disappointing as several other antimony preparations. Khalil (1926) reports favourably on the use of sodium antimony tartrate. He has also used antimosan, stibenyl, and urea stibamine, but refrains from drawing conclusions. Gordon (1926) reports favourably on the use of emetine periodide in the treatment of *S. haematobium* infections in children. This is a very expensive and rather unstable preparation.

Tootell (1923) is not favourably impressed with mercurochrome 220 soluble. Miyagawa (1926) has found stibnal and " neostibnal " effective in the treatment of *S. japonicum*. Stibnal is evidently sodium antimony tartrate and is acid in reaction, while the " neostibnal " is stibnal rendered slightly alkaline.

Cawston (1928) records good results with carbon tetrachloride. I have tried this drug on infected sheep, but without success. Shattuck and Willis (1928) report that antimony sodium thioglycollate and antimony thioglycollamide had favourable effects in cases of urinary schistosomiasis. They claim that these drugs are much less toxic than tartar emetic.

Christopherson (1928) is convinced that the only method of eradicating schistosomiasis from a given locality is to rid both snails and humans of the infection by irrigating the veins of the definitive host with tartar emetic. He observes that South Africa is adopting this line of action by treating the school children in the endemic areas.

(2) PREVENTING THE MIRACIDIA FROM REACHING THEIR HOSTS.

Blacklock (1925) has rightly remarked that endemic *schistosomiasis* is a sign of inadequate education in hygienic principles. The same holds in South Africa, where the insanitary disposal of human excreta is also responsible for the prevalence of *cysticercosis* in cattle and pigs. Sanitation in some of our villages leaves much to be desired.

In the life-cycle of the human schistosomes the miracidia can be successfully dealt with. In domestic stock the prevention of water-pollution with animal excreta would not be so easy. On some farms there are only a few snail-infested pools, and these should be fenced off. Municipalities should pass by-laws prohibiting the bathing and fishing in streams or rivers within the municipal boundary. The control of water-pollution should be more strictly conducted than is at present the case.

(3) CONTROL AND ERADICATION OF THE INVERTEBRATE HOST.

Before an attack on the snails is launched, the species responsible for the transmission of the worm must be conclusively proved, or much energy and money may be wasted. A careful study of the bionomics and the susceptibilities of the intermediate hosts may suggest the means by which they can be most readily eradicated.

Methods which have been suggested for the eradication of snails are :—

(i) *The Use of Chemicals lethal to Fresh-water Snails.*

Thomas (1883) suggests dressing the ground with lime or salt, or both, to destroy the snails responsible for the transmission of *F. hepatica*. Leiper (1915) suggests the use of chemical agents such as ammonium sulphate. Chandler (1920) observes that one part of copper sulphate to from 500,000 to 2,000,000 of water destroyed several species of snails within two days. Leiper (1922) notes that copper sulphate in the dilution 1 in 5,000,000 kills *Bulinus contortus* and *Planorbis boissyi*, while *Lymnaea truncatula* required a dilution of 1 in 2,000,000. Walton and Jones (1925) recommend the use of copper sulphate, in one-half per cent. dilution, against *Lymnaea truncatula*. They did not find ammonium sulphate as effective.

Other chemicals which have been successfully used are slaked lime and calcium cyanamid in concentrations of 0.1 per cent. Nagano (1926) found a 0.2 per cent. mixture of " lime nitrogen " efficacious against snails in Japan. Cawston (1928 *a*) states that the plant *Tephrosia macropoda* is highly toxic to *Physopsis* sp. and suggests that it should be utilized for eradicating the intermediate hosts of schistosomes. In a later communication he states that the active principle of the plant would appear to be about a hundred times more toxic than copper sulphate is for *Physopsis*.

I have tried copper sulphate in a dilution of about 1 in 2,000,000 on *Physopsis africana* var. *globosa* in a pool, and it killed all of them in eighteen hours. Thus far I have invariably found *Physopsis* in pools and backwaters in permanent collections of water. As in the Transvaal these collections are at their lowest in winter, it is evident that this is the time for poisoning the snails. At Humansdorp the flow in the river was lowest during the summer (the non-raining season). The non-rainy season should be selected for the attack on the snails.

(ii) *Subjecting the Snails to Sudden Drying.*

Leiper (1915) suggests killing off *Bulinus* by subjecting it to sudden drying in the irrigation canals. This mode of killing snails may be practical in irrigation canals in Egypt, but cannot be utilized in the schistosome-infected areas of the Union. *Lymnaea natalensis* may be controlled on liver-fluke infested farms by draining the marshy pastures.

(iii) *The Keeping of Domestic Ducks.*

Keatinge recommends the keeping of flocks of domesticated ducks for eradicating the snails. Cawston claims to have had good results with ducks. This mode of snail eradication needs closer investigation. There are hardly any experiments on record which prove the use of the domestic duck as a means of controlling the increase of the molluscs. My personal experience is that *Lymnaea natalensis* may flourish in the pools frequented daily by ducks.

(4) THE CERCARIAE AND THE DEFINITIVE HOST.

All pools known to be infested with schistosome cercariae should be avoided. In the case of the animal schistosomes, the pool or pools may be fenced off. If there is no other water available for drinking the stock, the water in one or more of the pools should be treated with copper sulphate to kill the intermediate hosts and the cycle thus arrested.

According to Leiper (1915), the cercariae can survive for only forty-eight hours. Where water is pumped from infected pools into tanks, it should not be used for two days. The use of "tabloids" of sodium bisulphate, 16 grains to a pint of water, is said to kill off the cercariae and renders the water safe for human consumption.

The campaign against schistosomiasis must be conducted energetically, and both the definitive and the intermediate hosts should receive due attention. It is undoubtedly a waste of public funds to treat school children while the adults and the molluscs roam about unattended. Khalil (1927) records the successful eradication of bilharziasis from an endemic area by the use of copper sulphate.

During a recent visit by some northern Transvaal farmers to this Institution it became evident that they knew little about the cause and prevention of urinary schistosomiasis. Most of them had never even seen the snail responsible for the transimssion of *S. haematobium*. Some of them questioned me for quite a time. One of the more elderly men ultimately asked me whether the medical practitioners did not know "all about it," or, if they did, why they did not tell them. Some of my experiences with medical practitioners prove that the farmer had reason to ask that question.

The endemic areas of the country are evidently flooded with leaflets dealing with the cause and spread of haematuria, but an ocular demonstration of the worms will impress the inhabitant more than the leaflets. The farmers should be made to realize that the present insanitary conditions on their farms leave much to be desired. The following quotation from a recent Editorial in the *Journal of the Medical Association of South Africa* is pertinent:—"We have tinkered with many subjects—with public health, medical supervision of schools and scholars, malaria prevention, medical service for rural communities, and many other matters—but we have not tackled one of these with energy, foresight, and the desire to make the public see that our point of view is, after all, the national point of view. In the future we must take our responsibilities much more seriously, subscribing to Larrey's formula that 'a physician is a priest who should preach and practise.'"

The same writer also makes the statement: "It has been said that we have larger sums of money devoted to animal research than to medical research."

The treatment of school children will, according to a recent newspaper announcement, be continued this year, but let us hope that due attention will be paid to the native and the snail.

I was recently told by a gentleman from Zululand that the charge for the course of treatment for schistosomiasis is £15. This is, according to the *Star* of Johannesburg, also the charge in some parts of the Transvaal. Very few of the poor whites can afford this, especially when they have more than one child infected. According to some reports, the control of *schistosomiasis* is of national importance. The disease is undoubtedly on the spread. This spread must be attributed to the movements of infected humans.

The practice of trekking with sheep about the country in times of drought has been forcibly demonstrated as a means of disseminating helminths. During the last serious drought several flocks were grazed in the endemic area in the Humansdorp District. Most of these flocks, fortunately, returned to areas claimed to be free of molluscs.

THE SOUTH AFRICAN FRESH-WATER SNAILS RESPONSIBLE FOR THE SPREAD OF TREMATODE DISEASES.

Photographs of various South African freshwater molluscs are included for the information of those interested. I would like to point out that the distribution of molluscs in the Union of South Africa is very imperfectly known and that our field colleagues can supply much of the information wanted. The bionomics of the various species needs further study and should receive the attention of the man on the spot.

Live snails may be forwarded to this Laboratory for identification, by animal experimentation, of the cercariae present. The snails, packed in slightly moist water plants, travel well by post or passenger train.

The species photographed are named as identified for us. It must be pointed out that the leading malacologists seem unable to reach an agreement with regard to the nomenclature and the definition of the genera and the species. Generic names of African molluscs such as *Bulinus* and *Lymnaea* are often misspelt as *Bullinus* and *Limnaea*. Let us hope that the malacologists and the International Commission of Zoological Nomenclature will soon rectify matters.

The freshwater molluscs of South Africa need revision badly, but such revision can only be attempted when the material is available.

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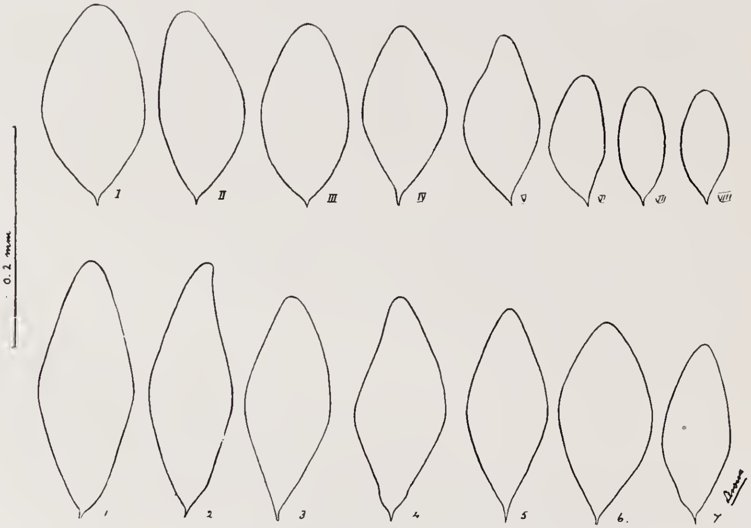
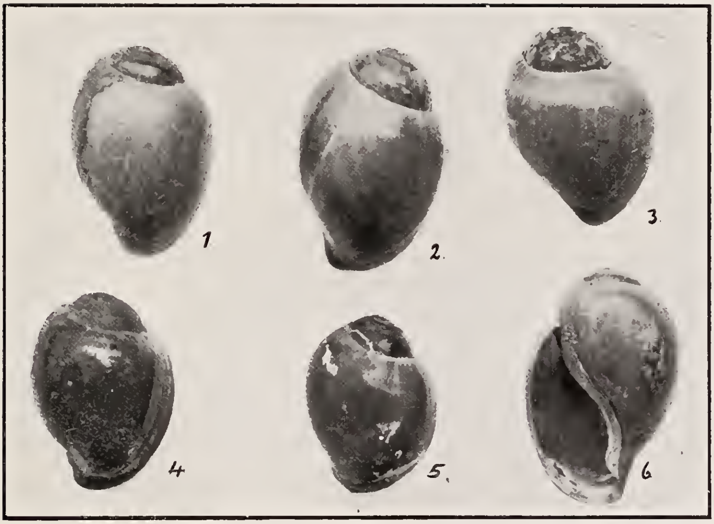
PLATE 1.



Figs. I to VIII.—*Schistosoma haematobia* ova from the urine of a human in Pretoria, Transvaal.

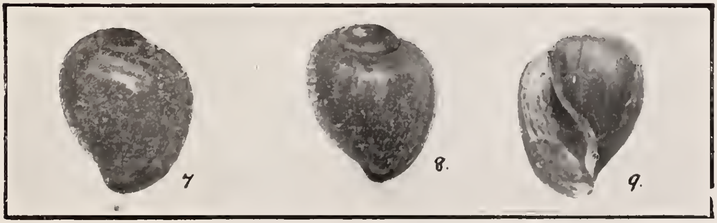
Figs. 1 to 7.—*Schistosoma mattheei* ova from the faeces of sheep from Humansdorp, Cape Province.

Figs. 1 to 6.

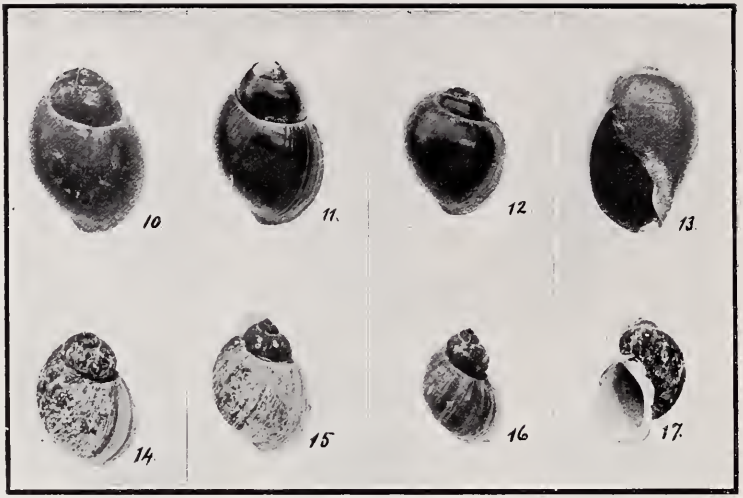


Specimens of *Physopsis africana*, var. *globosa* (Morelet), from Humansdorp, Cape Province. Magn. 1¾×.

Figs. 7 to 9



Specimens of *Physopsis conicum* from the Pretoria District, Transvaal. Magn. 1¾×.



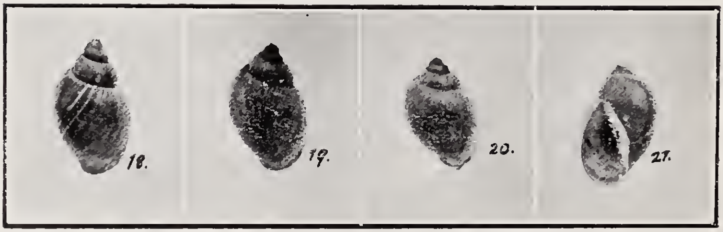
Figs. 10 to 13.

Specimens of *Physopsis africana* (Krauss) from Natal. Note the difference in the shape of Nos. 10 and 11 and Nos. 12 and 13. Magn. 1½×.

Figs. 14 to 17.

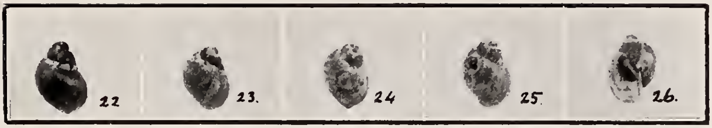
These are said to be specimens of *Bulinus tropicus* (Krauss). From the Pretoria District, Transvaal. Magn. 1½×. (See Figs. 32 and 33.)

Figs. 18 to 21.



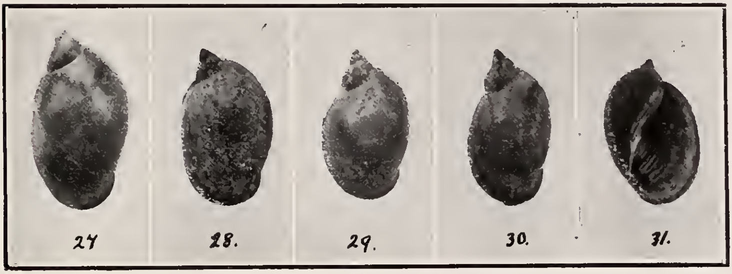
A fresh-water snail from the field cornetcy of Goudini, Worcester Cape Province. This species was found infected with cercariae of *Fasciola* and *Paramphistomum*. Mag. 1½ ×.

Figs. 22 to 26.



Immature (?) specimens of *Bulinus* from a vlei near Humansdorp. Mag. 1½×.

Figs. 27 to 31.

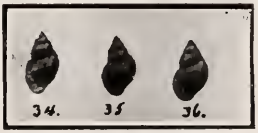


Specimens of *Lymnaea natalensis* (Krauss) from Humansdorp. Magn. 1½×.

Figs. 32 and 33.

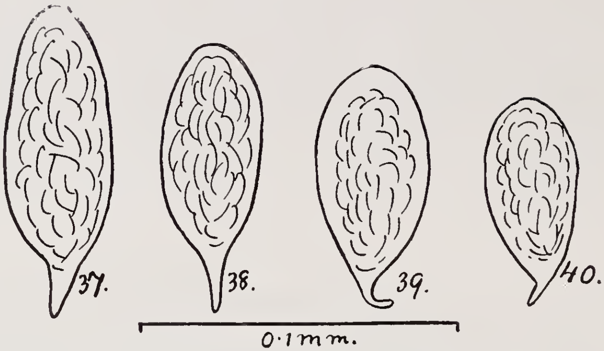
Specimens of *Bulinus* identified as *B. schackoi* (Jickeli), which species is, according to Cawston (1928), identical with *B. tropicus*, *B. natalensis*, *B. craveni*, and *B. sericinus*.

Figs. 34 to 36.



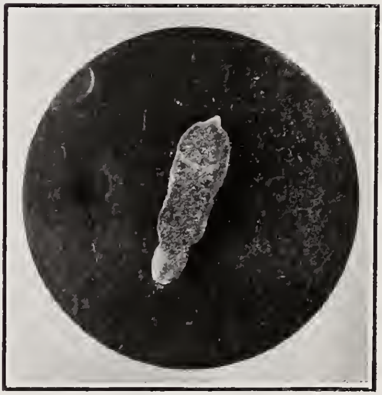
Specimens of *Lymnaea truncatula* (Müller), a common intermediate host of *Fasciola hepatica* in the Western Province, Cape. Magn. 1½×.

FIGS. 37 TO 40.



Eggs from the uterus of *S. mattheei*, which had been subjected to tartar emetic *in vivo*.

FIG. 41.



A specimen of *Fasciola hepatica* from the gall-bladder of a sheep treated with carbon tetrachloride. Note the degeneration of the posterior three-quarters of the fluke.

*15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.*

Two Species of *Haemonchus* Cobb, 1898, Parasitizing the Camel in the Cape Province.

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

RECENTLY the writer had the opportunity of examining specimens of *Haemonchus* collected from the abomasum of a camel (*Camelus dromedarius*) destroyed at this Institution on arrival from Vryburg, Cape Province. This camel had run for some years with cattle on the farm Armoedsvlakte owned by the Division of Veterinary Services.

The microscopic examination of the few males collected revealed the presence of two species of *Haemonchus* (*H. longistipes* and *H. contortus*).

As *H. contortus* has not previously been recorded from this host, the specimens were examined closely, but could not be differentiated from members of this species collected from cattle and sheep. The spicules were identical with those of the type species of this genus as is evident from Table I.

The larger females of *H. contortus*, owing to the presence of a rather small linguiform process not uncommonly met with in specimens from cattle, could not be too readily differentiated from the specimens of *H. longistipes* of approximately the same size. The most important measurements of these specimens of *H. contortus* are given in Table II.

The measurements (Tables III and IV) of the specimens of *H longistipes*, Railliet and Henry, 1909, collected agree with the findings of Boulenger (1921).

I have nothing to add to Boulenger's description except that in the specimens examined by me, the ventral lip of the cloaca ends in an appendage (Fig. 10) and is not as bluntly rounded off as figured by him (1921, Fig. 2a).

As this appendage is frequently bent dorsally, it is quite readily missed unless the lip is viewed laterally.

The short dorsal lip of the cloaca is, as in *Haemonchus contortus* (Fig. 2), supported by two rays.

TABLE I.

The Measurements (in microns) of the spicules of H. contortus *from the camel compared with those of* H. contortus *from sheep and from cattle.*

Authors		Ransom, 1911.	Dykmans, 1921.	Travassos. 1921.	Veglia, 1916.	Le Roux.		
Locality		North America.	North America.	South America.	South Africa.	South Africa.		
Origin		Sheep and Cattle.	Cattle.	Sheep and Cattle.	Sheep and Cattle.	Camel.	Sheep.	Cattle.
Length of Spicules		300–500	400–430	390–500	460–470	494–504	462–506	462–495
Barb-like projection from the posterior extremity of spicule	Right	40	28–32	28–40	40	49–58	40–60	48–60
	Left	20	14–17	14–20	20	23–25	20–28	26 30

TABLE II.

The Measurements (in millimetres) of six females of H. contortus *from the camel.*

Length of worm	16·0	17·5	17·8	18·0	18·0	23·0
Maximum diameter of body	0·29	0·31	0·33	0·37	0·329	0·349
Length of oesophagus	1·50	1·60	1·63	1·56	1·60	1·66
Maximum diameter of oesophagus	0·104	0·120	0·112	0·120	0·112	0·108
Anterior extremity to excretory pore	0·33	0·32	0·33	0·316	0·32	0·34
Anterior extremity to cervical papillae	0·40	0·40	0·40	0·38	0·42	0·42
Vulva to anus	2·50	3·10	3·10	3·28	2·90	3·72
Anus to tip of tail	0·48	0·56	0·54	0·66	0·60	0·82
Length of linguiform process	0·14	0·16	0·12	0·20	0·14	0·20
Measurements of eggs in utero	0·070 × 0·038	0·068 × 0·038	0·072 × 0·038	0·076 × 0·040	0·075 × 0·040	0·078 × 0·042

TABLE III.

The Chief Measurements (in millimetres) of five females of Haemonchus longistipes, *Railliet and Henry*, 1909, *from the camel* (Camelus dromedarius) *in the Cape Province, compared with those as recorded by Railliet and Henry* (1909) *and Boulenger* (1921).

Authors	Railliet and Henry, 1909.	Boulenger, 1921.	Le Roux.				
Locality	Africa and India.	India.	Cape Province, South Africa.				
Length of worm	26–29	23–35	28·5	29·0	31·5	31·5	32·0
Maximum width	—	0·45–0·65	0·595	0·595	0·627	0·680	0·653
Length of oesophagus	—	1·60–2·10	1·810	1·800	1·940	1·940	1·940
Maximum width of oesophagus	—	—	0·168	0·156	0·160	0·176	0·183
Anterior extremity of excretory pore	—	—	0·281	0·400	0·356	0·324	0·356
Anterior extremity to cervical papillae	—	0·39–0·53	0·399	0·518	0·448	0·432	0·443
Vulva to tip of tail	—	4·5 –6·8	6·0	5·75	6·5	6·0	6·0
Anus to tip of tail	—	0·47–0·58	0·454	0·432	0·540	0·486	0·486
Caudal papillae to tip of tail	—	—	0·108	0·130	0·160	0·160	0·140

TABLE IV.

The Chief Measurements (in millimetres) of six males of Haemonchus longistipes, *Railliet and Henry*, 1909, *from the camel* (Camelus dromedarius) *in the Cape Province, compared with those recorded by Railliet and Henry, and Boulenger.*

Authors.	Railliet and Henry, 1909.	Boulenger, 1921.	Le Roux.					
Locality.	Africa and India.	India.	Cape Province, South Africa.					
Length of worms.	20–21	18–25	20	21	22	22	23	23
Maximum width of worms.	—	0·4	0·400	0·389	0·378	0·378	0·389	0·450
Anterior extremity to excretory pore.	—	—	0·432	0·432	0·380	0·540	0·486	0·464
Anterior extremity to cervical papillae.	—	0·39–0·53	0·580	0·580	0·514	0·680	0·670	0·660
Length of oesophagus.	—	1·6 –2·1	1·746	·1649	1·804	1·649	1·649	1·552
Maximum width of oesophagus.	—	—	0·156	0·164	0·121	0·156	0·160	0·132
Length of spicules.	0·625	0·60–0·65	0·66	0·63	0·62	0·62	0·62	0·66
Barb to extremity of right spicule.	—	0·09–0·10	0·092	0·094	0·090	0·996	0·090	0·098
Barb to extremity of left spicule.	—	0·038–0·04	0·043	0·043	0·038	0·042	0·040	0·044
Length of common stem of dorsal ray.	—	—	0·213	0·194	0·183	0·183	0·168	0·168
Length of the two main brabches of dorsal ray	—	—	0·066	0·066	0·078	0·086	0·092	0·096
Length of the gubernaculum.	—	0·3 –0·33	0·324	0·324	0·324	0·326	0·324	0·324
Width of the gubernaculum.	—	—	0·119	0·119	0·119	0·119	0·119	0·119

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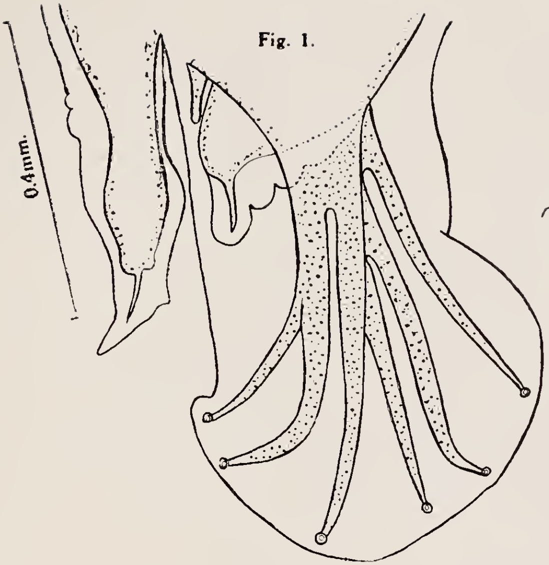

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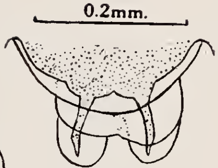
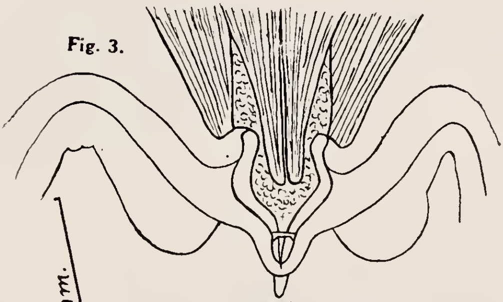
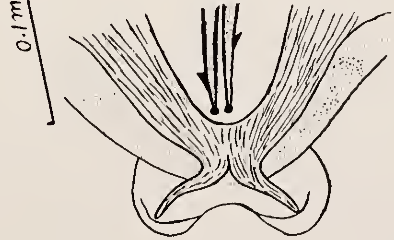


Fig. 2.

Fig. 3.





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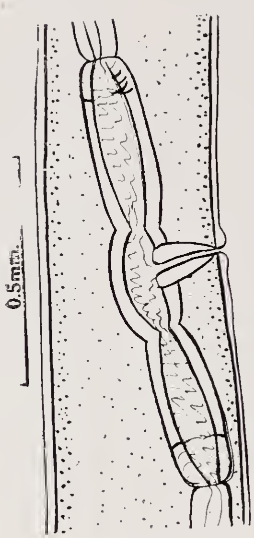

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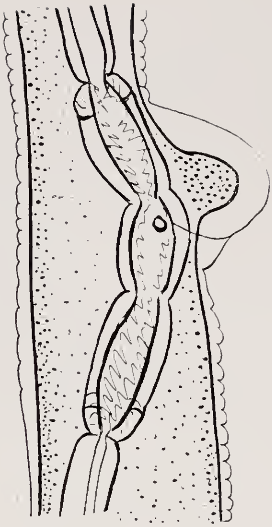
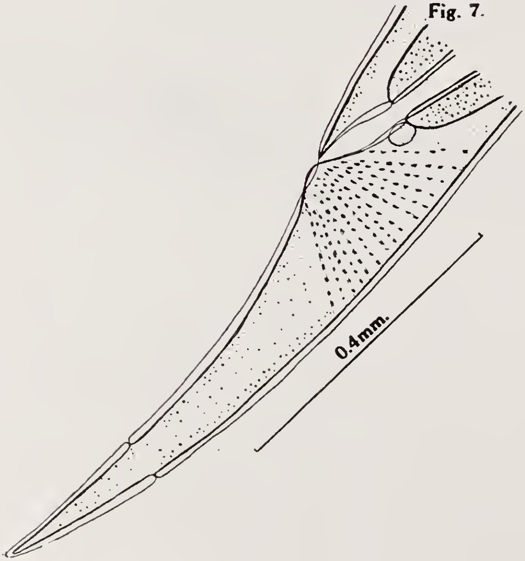
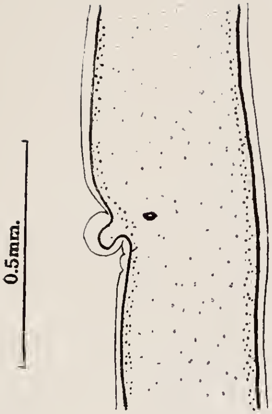


Fig. 6.

Fig. 7.





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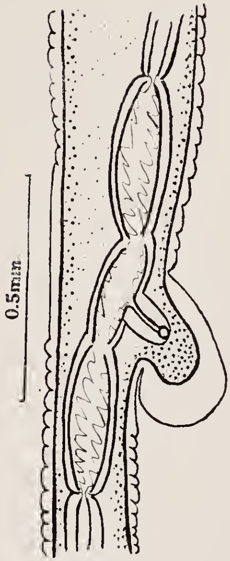

Fig. 9.

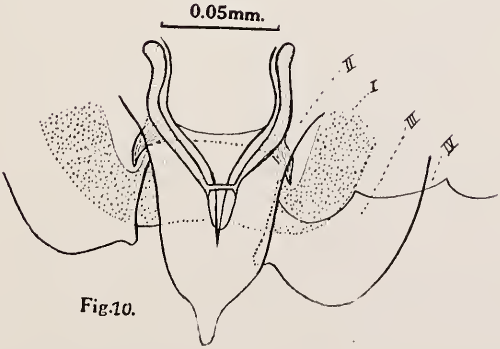

Fig. 10.

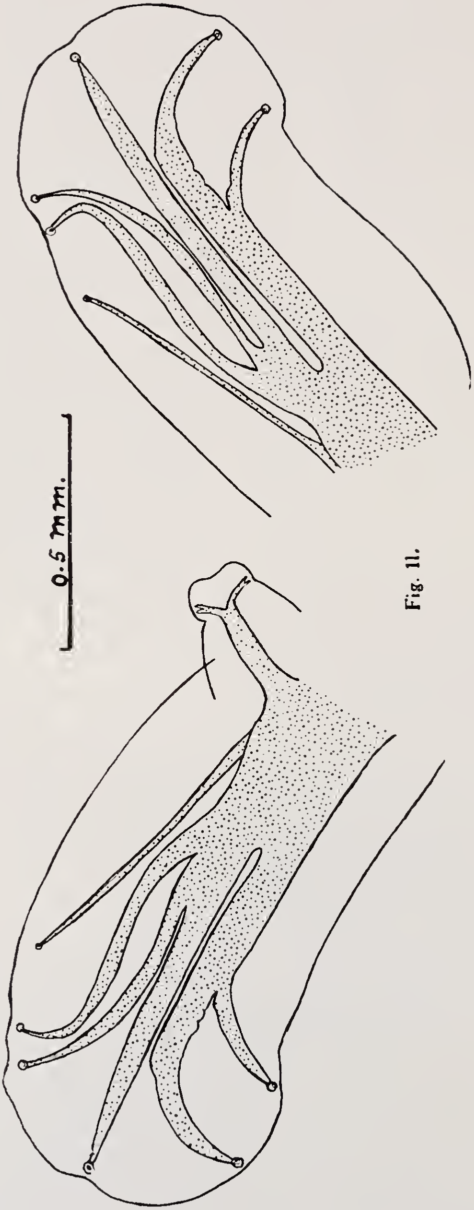

Fig. 11.

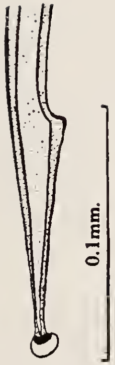

Fig.13.

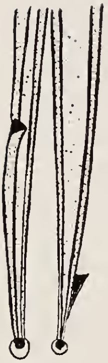

Fig.14.

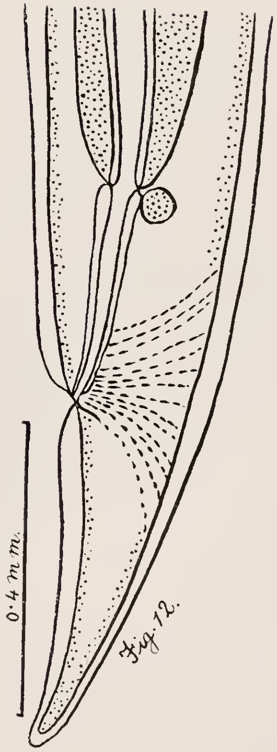

Fig. 12.

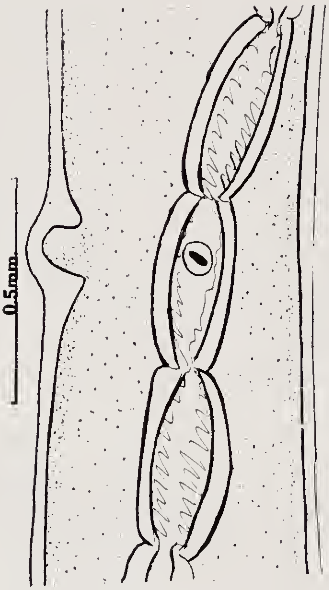

Fig.15.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

A Preliminary Report on Three New Members of the Genus *Haemonchus*, Cobb, 1898, from Antelopes in South Africa.

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

About three years ago Mr. D. T. Mitchell, M.R.C.V.S., Sub-Director of Veterinary Services, presented me with some nematodes collected from the *abomasi* of two eland (*Taurotragus oryx*, Pall.) shot in the Drakensberg National Game Reserve, Natal.

The examination of the material, preserved in 10 per cent. formalin, revealed the presence of two species of nematodes, *Ostertagia circumcincta* and a species of *Haemonchus* new to science. For this species the name *Haemonchus mitchelli*, sp. nov., in honour of the donor, is designated.

On my transfer to the Onderstepoort Veterinary Research Laboratory, I had the opportunity of examining three lots of *Haemonchus contortus* (Rud.) contained in the helminthological collection and labelled as having been collected from the *abomasi* of the Cape koodoo (*Strepsiceros strepsiceros* Pall.), the blue wildebeest (*Gorgon taurinus* Burch.), and the African buffalo (*Syncerus caffer*, Sparrm.).

The examination of the spicules of the specimens decolorized in lacto-phenol, proved them not to be members of the type species as shown on the labels.

For the species from the abomasum of the Cape koodoo, I designate the name *Haemonchus vegliai*, sp. nov., in honour of Dr. Francesco Veglia, whose work on the morphology and life-history of *Haemonchus contortus* is well known to all interested in Nematology.

The specimens from the blue wildebeest and the African buffalo were identical, judging from the spicules. For this species the name *Haemonchus bedfordi* in honour of the donor, Mr. G. A. H. Bedford, Research Officer in Entomology, is designated.

As type host of this species, I select the blue wildebeest, from which the specimens collected by Mr. Bedford were most excellently preserved in glycerine alcohol.

The specimens from the African buffalo were collected in Zululand by Dr. H. H. Curson while investigating Nagana there. This material was not well preserved. The measurements given for *H. bedfordi* are of specimens taken from blue wildebeest shot in the Waterberg district, Northern Transvaal.

Most of the material from the koodoo was not well preserved and the measurements given below cannot be taken as absolutely characteristic for the species.

A detailed description of each individual species is held over. The three species are typical members of the genus *Haemonchus*, Cobb, 1898. The differences which allow of their identification are given below, and their chief measurements are tabulated in Tables I and II.

It must be pointed out that only the most perfectly preserved specimens were examined and that twelve males and twelve females of each species were measured.

Differentiation of the species.

Here, as in the genus *Trichostrongylus*, Looss, 1905, the examination of males is essential for the identification of a species.

The males of the different species are readily distinguished from each other by the characters of their spicules.

The tips of the spicules do not only bear characteristic barbs, but these barbs are situated at constant levels, varying with the different species. In the case of *H. vegliai*, the left spicule is furnished with two barbs (Figures 21 and 22). The lobes and their rays comprising the *copulatory bursa* show slight variations in the different species.

Variations are also evident in the genital cones of various species. Of all the characters referred to, those of the spicules alone are of such a nature as to allow of the differentiation of the species.

The females could to some degree be recognized by the characters of their *linguiform processes*. As in the type species, the *linguiform process* is liable to considerable variation within specimens from the same host.

In *H. mitchelli* the *linguiform process* is a fairly stout and well-developed appendage situated in front of the genital opening and partly or wholly overlapping it. In the majority of specimens the vulva with the vagina slightly evaginating is located at the right commissure, formed by the *linguiform process* and the body.

In *H. bedfordi* the linguiform process shows still more anomalies. In some specimens there are well-developed *pre-vulvar* and *post-vulvular processes* (*linguiform processes*). In others there are only post-vulvular processes, and these were often at a considerable distance from the genital opening.

In a third group there were no *linguiform processes present.*

In all three groups one or two *lateral cuticular swellings* may be absent or present in the region of the vulva. These swellings are associated with sexual functions, and in specimens observed in copula they were gripped by the lateral lobes of the copulatory bursa.

H. vegliai yielded variations as well. In most specimens the *linguiform process* was replaced by a mere swelling as figured.

The apparent anomalies of the vulvular linguiform process as met with in *Haemonchus contortus* has been commented on by Veglia (1915) and Dikmans (1921). Judging from the observations of Veglia and Dikmans on the type species and my personal findings, just given, it is evident that the character of this process, in any one known species, is not so constant as to accept it as of a specific generic character.

Baylis and Daubney (1922) differentiated the species *Haemonchus cervinus* of the spotted deer (*Cervus axis*) from *Haemonchus contortus* because " the vulva is slightly prominent, but possesses no overhanging anterior lip such as is found in *H. contortus*." Specimens of *H. contortus* without an overhanging linguiform process are by no means uncommon from both sheep and cattle in South Africa.

The species *Haemonchus cervinus* Baylis and Daubney (1922), should most probably be considered a *species inquirendae*. An examination of the spicules (if present) of the damaged male is desired if *H. cervinus* Baylis and Daubney, 1922, is to be retained as a valid member of the genus.

Curson (1928), recording *Haemonchus contortus* (Rud.) from the ox, sheep, goat, buffalo and wildebeest in Zululand, comments on the fact that although the wild ruminants were heavily infected, they showed no ill effects such as are manifested by domestic stock similarly infested.

Curson's record of *H. contortus* from the buffalo and the wildebeest is incorrect, as is proved by the specimens collected by him and deposited in the helminthological collection at Onderstepoort.

Haemonchus contortus (Rud.) of Curson 1928 now becomes a synonym of *Haemonchus bedfordi*, sp. nov.

Although the two old eland bulls shot by Mr. Mitchell were very heavily infected with the two species mentioned elsewhere, they were in excellent condition. Their age and the good grazing probably accounted for the absence of lesions attributable to *Haemonchosis*.

TABLE I.

Measurements (in millimetres) of the males of Haemonchus contortus, H. mitchelli, H. vegliai *and* H. bedfordi.

Species		*H. contortus.*	*H. mitchelli*, sp. nov.	*H. vegliai*, sp. nov.	*H. bedfordi*, sp. nov.
Hosts		Sheep, goat, cattle, camel.	*Taurotragus oryx* Pall.	*Strepsiceros strepsiceros* Pall.	*Synceros caffer* Sparrm., *Gorgon taurinus* Burch.
Length of body		15–18	10·5 –14	9·5 –11	9·8 –11·5
Maximum breadth of body		0·27	0·27 –0·33	0·24 –0·3	0·23 –0·27
Anterior end to excretory pore		0·276	0·20 –0·30	0·20 –0·25	0·21 –0·32
Anterior end to cervical papillae		0·32 –0·44	0·28 –0·43	0·30 –0·40	0·33 –0·40
Maximum width of oesophagus		0·15	0·12 –0·13	0·11 –0·13	0·09 –0·14
Length of oesophagus		1·50	1·25 –1·42	1·10 –1·60	1·10 –1·30
Length of spicules		0·460–0·506	0·50 –0·54	0·44 –0·48	0·40 –0·46
Distance of barbs from tip of spicules	Right	0·040–0·060	0·07 –0·086	0·06 –0·07	0·09 –0·11
	Left	0·020–0·030	0·034–0·040	(i) 0·040–0·050 (ii) 0·028–0·035	0·044–0·053
Length of gubernaculum		0·20 –0·25	0·26 –0·37	0·28 –0·35	0·25 –0·32

TABLE II.

Measurements (in millimetres) of females of H. contortus, H. mitchelli, H. vegliai *and* H. bedfordi.

Species	*H. contortus.*	*H. mitchelli*, sp. nov.	*H. vegliai*, sp. nov.	*H. bedfordi*, sp. nov.
Length of Body	16–24	18·5–21·0	14·5–20	14·5–19·5
Maximum breadth of body	0·29–0·40	0·40–0·46	0·34–0·42	0·33–0·38
Length of oesophagus	1·50–1·70	1·60–1·73	1·20–1·70	1·20–1·49
Maximum width of oesophagus	0·11–0·13	0·17–0·20	0·17–0·21	0·12–0·17
Anterior extremity of excretory pore	0·27–0·34	0·20–0·29	0·21–0·30	0·25–0·31
Anterior extremity to cervical papillae	0·32–0·42	0·29–0·38	0·34–0·49	0·29–0·53
Vulva to posterior extremity	3·50–5·10	3·20–3·57	3·00–3·80	2·90–3·70
Anus to posterior extremity	0·40–0·63	0·29–0·38	0·31–0·59	0·33–0·42

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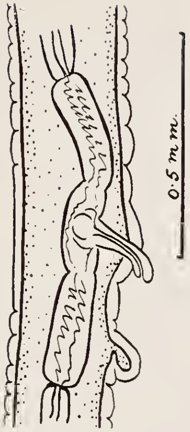
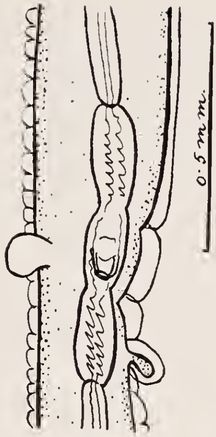


Fig. 1.



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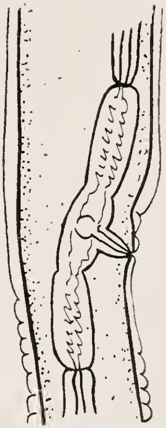

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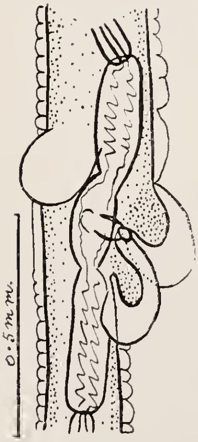

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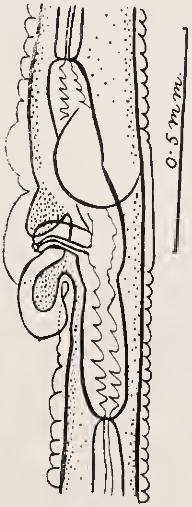

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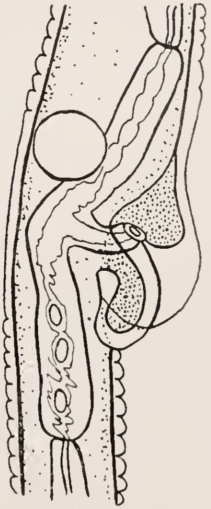

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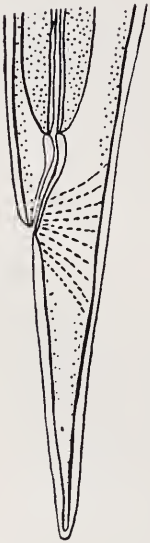

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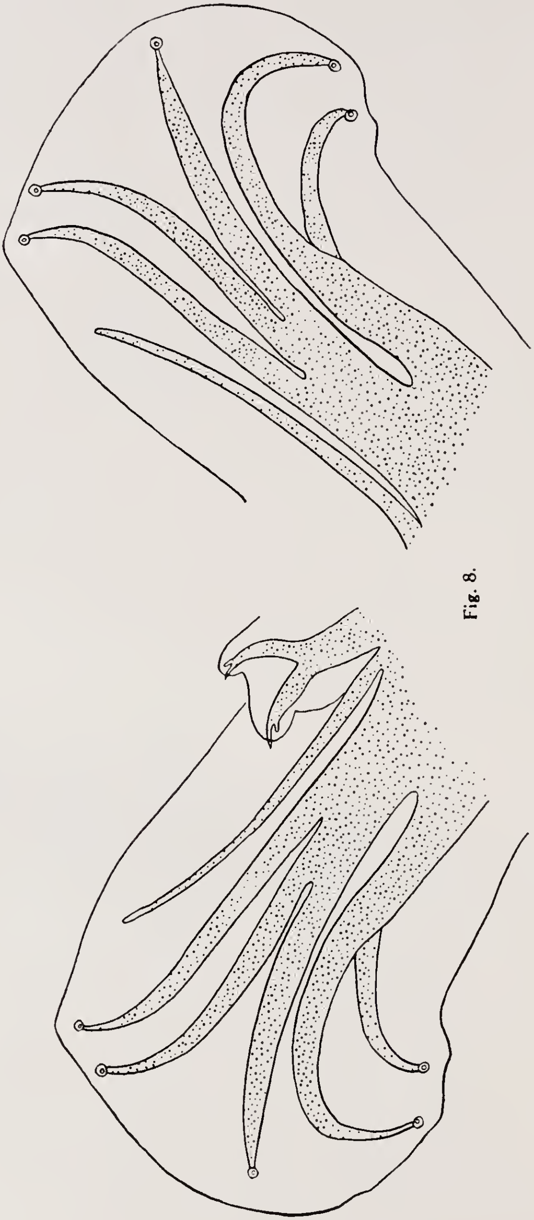

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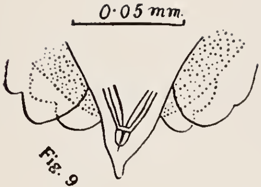

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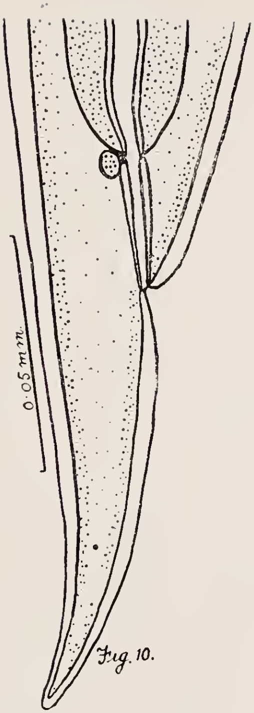

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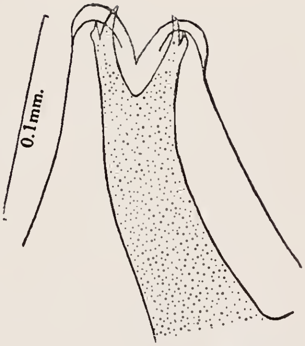

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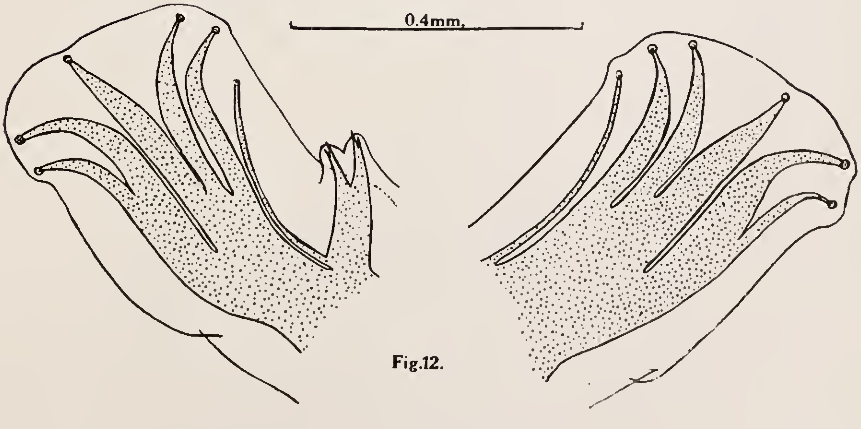

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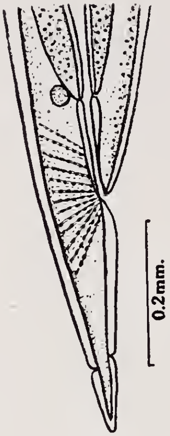

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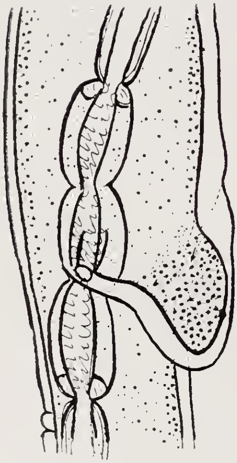

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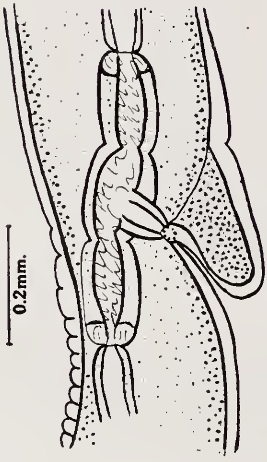

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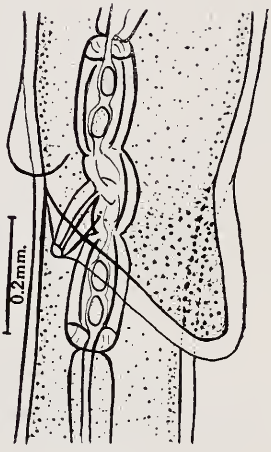

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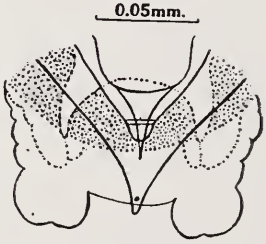

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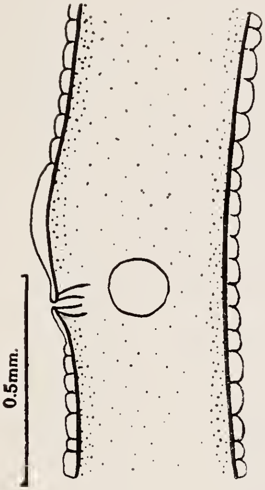

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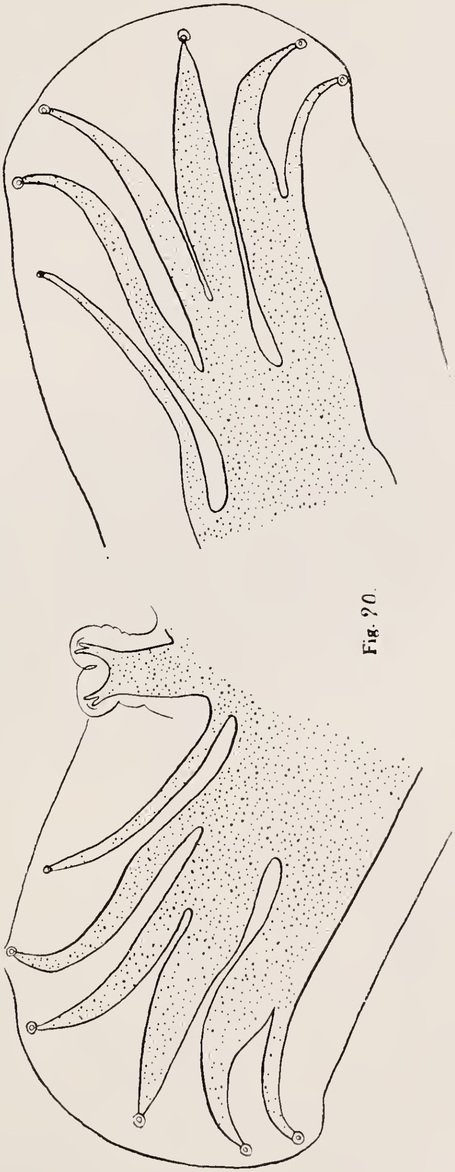

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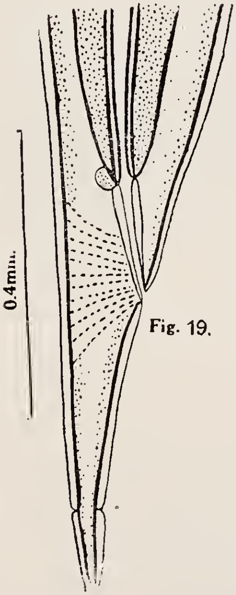
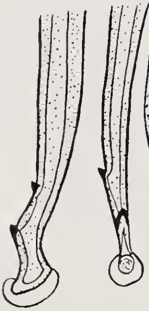


Fig. 19.

Fig 21.



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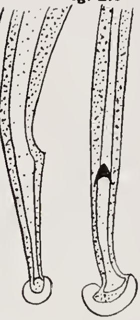

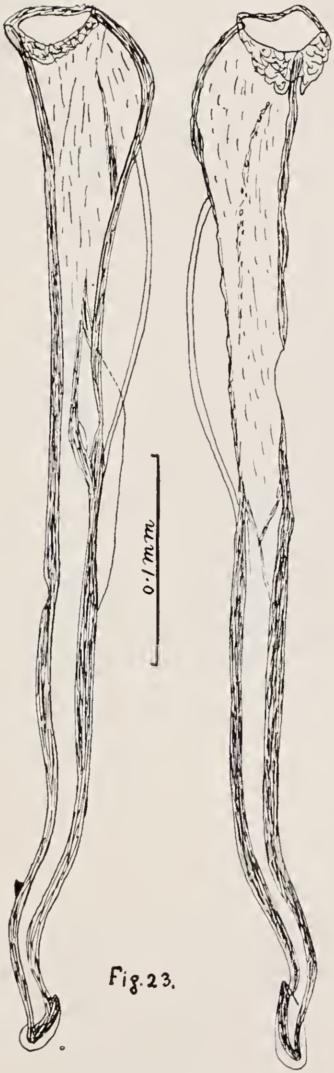

Fig. 23.

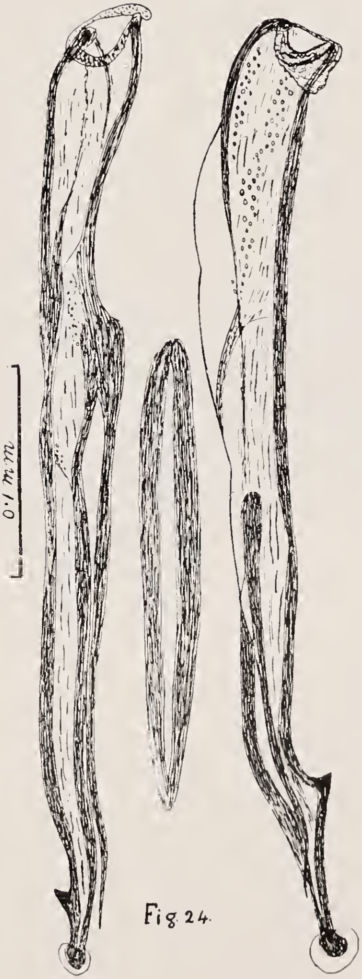

Fig. 24.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

On an Oesophagostome *(Oesophagostomum susannae,* sp. nov.) from the Springhare *(Pedetes caffra),* together with Remarks on closely related Species.

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

In 1927 the writer collected four nematodes from four haemorrhagic submucosal cysts in the caecum of a Springhare shot at the Nooitgedacht Veterinary Research Laboratory, Ermelo, Eastern Transvaal. The search of the lumen of the alimentary tract for helminths yielded a solitary female of the genus *Trichocephalus*, located in the caecum.

The microscopic examination of the specimens from the cysts proved them to be sexually immature adult members (two males and two females) of the genus *Oesophagostomum* Molin, 1861. The structure of the buccal capsule and the character of the oesophageal funnel showed that they were members of a species closely related to three or possibly four of the known oesophagostomes. In Table I the chief measurements of the new species will be found given with the available measurements of the related forms.

Before dealing with the morphology of *O. susannae*, the related species will be considered briefly as regards hosts and geographical distribution.

Oesophagostomum apiostomum (Willach, 1891), Railliet and Henry, 1905, was originally described as *Sclerostoma apiostomum* from nodules in the intestine of *Macacus cynomolgus.* Weinberg collected several specimens of this species from *M. cynomolgus* and *M. sinicus.* It is on this material that Railliet and Henry base their description of *O. apiostomum.*

Leiper (1911) declares *O. brumpti*, Railliet and Henry, 1905, to be synonymous with *O. apiostomum*, which he records:

(*a*) from the great intestine of monkeys in Uganda;
(*b*) from cysts in the wall of the colon of *M. sinicus*; and
(*c*) as passed by a native patient of Dr. Andrew Foy at Ibi, Northern Nigeria.

Leiper (1913) once more declares *O. brumpti* as synonymous with *O. apiostomum*, which is this time recorded from inmates of the Zungeru Gaol, Northern Nigeria. His drawing (Fig. 15, p. 274) of the anterior extremity of the worm appears without the recurved oesophageal teeth characteristic of the members of the subgenus *Conoweberia* Ihle, 1922. Judging from the measurements given by Leiper (1911 and 1913), I am inclined to agree with Railliet and Henry (Brumpt 1927, p. 674), and Ihle, 1922, that Leiper was dealing with a species or species other than *O. apiostomum* or *O. brumpti*. A re-examination of Leiper's material seems desirable.

Lane and Low (1923, 1899), tabulating the chief measurements of the Strongylidae rarely infesting man, has the names *O. apiostomum* and *O. stephanostomum* interchanged. They have adopted *O. brumpti* as a synonym of *O. apiostomum*, for whom only Africa is cited as locality. Walker (1913) records *O. apiostomum* as of common occurrence in the Philippines. Ihle (1922) gives the most detailed description existing for *O. apiostomum*, which is recorded for the first time in the wall of the intestine of an orang-outan.

Until Leiper's *O. apiostomum* is definitely proved to be identical with that of Willach, the distribution of this species remains confined to Asiatic monkeys.

Oesophagostomum brumpti Railliet and Henry, 1905, was originally described from material collected by Brumpt from nodules in the intestinal wall of a negro in the vicinity of the River Omo. Smit (1919) records this species from a monkey in Java. His description of the parasite is so incomplete that the identity is not proved. He gives the length of the spicules as 1300 μ, which seems to indicate the possibility of the parasite being *O. apiostomum*.

Henry and Joveux (1920) record *O. brumpti* as a parasite of man, *Troglodytes niger*, *Cercopithecus callitrichus*, *C. patas*, and *Papio sphinx* in Upper French Guinea.

Joyeux (Brumpt 1922) saw specimens of this species passed by natives after the administration of thymol.

Oesophagostomum xeri Ortlepp 1922 was obtained from the caecum and caecal wall of the bristly ground-squirrel (*Geosciurus capensis*, syn. *Xerus setosus*), from South Africa, where this host is found living in close association with the springhare, as it not infrequently is.

Oesophagostomum susannae can be readily distinguished from the above-mentioned species by the possession of the characteristic short, thick, and dorsally tilted, blunt tail of the female.

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The worms extracted by puncturing the cysts were of a slightly dirty white colour. The body fluid of the cephalic extremity, in the immediate neighbourhood of the oesophagus, was tinted slightly red, as is often observed in members of the genus *Trichonema*—inhabitants of the large intestines of equidae. No curving of the cephalic extremity, a characteristic of oesophagostomes furnished with lateral cervical alae, occurred in these specimens on preservation in hot glycerine alcohol. Neither was this observed in sexually mature specimens collected for me by my colleague, Mr. I. P. Marais, and preserved in cold 10 per cent. formalin.

Both males and females showed slight attenuation towards the extremities. This attenuation was in the female more noticeable at the cephalic end, whereas in the male it was more marked at the posterior extremity.

The anterior extremity inflated to form a prominent *mouth collar* and limited posteriorly by a conspicuously deep *cephalic groove,* is ornamented with the usual six *circum-oral papillae.* In most of the specimens preserved in formalin the mouth collar was not so markedly inflated and hence had a less deep *cephalic groove.* In these specimens the *sub-dorsal* and the *sub-ventral cephalic* papillae showed up prominently, often projecting 33 μ beyond the anterior surface of the mouth collar. These papillae appear with distinctly constricted off, lanceolated tips measuring approximately 5 μ in length with a diameter of 3 μ. In specimens with markedly inflated mouth collars, the papillae appear slightly shorter owing to the pushing forward of the cuticle enveloping the cores of the papillae.

The *lateral cephalic papillae* appear as broad flat circular papillae on the antero-lateral surface of the mouth collar. The papillae are, in the cases with inflated mouth collars, situated in a depression of the cuticle. In other cases they project slightly beyond the surface of the cuticle.

The *cervical vesicle,* well marked in the sexually immature adults from the submucosal cysts and preserved in hot glycerine alcohol, extends from the cephalic groove to the level of the cervical papillae. In the specimens obtained from the lumen of the caecum and preserved in cold 10 per cent. aqueous solution of formalin, the *cervical vesicle* was much less prominent. In a few cases it appeared practically non-existent. In these latter, folds, suggesting escape of fluid from the vesicle, were numerous. This observation is recorded to warn workers aganist attaching too much significance to the degree of development of such easily distorted structures as the *mouth collar, cephalic groove,* and *cervical vesicle.* The nature of these is too readily affected by different methods of preservation to be of any specific generic significance. Thornton (1924), in his description of *Oesophagostomum oldi,* Goodey 1924, notes that the specimens examined by him at Liverpool had but slightly developed cephalic vesicles, with three to four lesser circular grooves, not recorded by Goodey, anterior to the cervical groove. As the worms were excellently preserved, Thornton did not regard their presence as due to shrinking of the cuticle. Authors unfortunately do not always state how the specimens examined by them were orginally preserved.

The degree of inflation of the cervical vesicle is in the species *O. radiatum* influenced by the activity of the parasite at the time of collection. In specimens with cephalic extremities embedded in crypts in the mucosa the vesicles were much more inflated than in those found free in the lumen of the gut. This inflation in the case of *O. radiatum* is an adaptation for anchoring itself in mucosal crypts, while it feeds on blood (?) or body tissues (?) acted on by secretions from the cephalic and oesophageal glands. The lumens of the older crypts were almost invariably filled with a coagulated dirty brown mass—material similar to that lining the ulcers caused by *Triodontophorus tenuicollis* in the right dorsal colon of equines.

Ventrally and on a level with the *cervical nerve ganglion* the cuticle of the cervical vesicle is indented to form the *ventral cervical groove*, in the posterior wall of which the *excretory pore* is localized.

The *cervical papillae* are prominent and in general disposition not unlike those of the type species of the genus. In a few individuals the free attenuated portion was wholly or partially missing on one or both sides.

The *cephalic*, the *cervical* and the *subventral oesophageal glands* are arranged as described by Goodey (1924) for *O. dentatum*.

The dorsal oesophageal gland opens into the buccal cavity at the summit of a well-developed *dorsal gutter*, a structure hitherto undescribed for oesophagostomes. The species closely related to the one under discussion should be re-examined for this structure, which was found to measure approximately 6-7 μ in height by 5 μ in width.

The *oral aperture* situated centrally on the anterior extremity is guarded by twelve stout, long (20 μ), sharply pointed elements composing the *external leaf crown*.

The buccal cavity is circular with stout, thick, cuticularized wall. Posteriorly the wall tapers to a slightly sharper edge than anteriorly and articulates with the oesophagus, which is anteriorly slightly cuticularized and grooved to receive the cuticularized wall of the buccal capsule. Dorsally the depth of the buccal capsule is 13 μ, whilst ventrally it is 16-17 μ. Medially and anteriorly the wall of the buccal cavity bears a prominent cuticularized fold, projecting medially and slightly anteriorly. At this point the lumen of the cavity is reduced to 33 μ, while the internal diameters at the inlet and the outlet of the capsule are 46 μ and 50 μ respectively.

In some of the specimens examined there was a distinct internal leaf-crown composed of rather minute elements arising from the cuticularized fold referred to above. The apparent absence of an internal leaf-crown in other specimens can only be attributed to the diminutiveness of its elements and the fact that they are obscured by the thick (5-6 μ) wall of the buccal cavity.

The cuticular fold described above is figured also for *O. apiostomum* by Ihle (1922). He does not express an opinion as to its function or its analogy to an interval leaf-crown which he declares seems absent.

For *O. xeri* Ortlepp (1922) there is described an internal leaf-crown of rather small elements.

Posterior to the chitinous fold the surface of the buccal wall is lined by a prominently inflated cuticle.

The buccal capsule leads posteriorly into a deep (36 μ) and wide (30 μ) oesophageal funnel formed by an increase in width and a decrease in thickness of the anterior extremities of the three oesophageal sectors. The cuticular lining of the lumen of the oesophagus and oesophageal funnel, instead of being continued to end in acute teeth projecting into the buccal cavity, is at the posterior border of this structure deflected medially and posteriorly to form a *dorsal* and two *subventral oesophageal teeth*.

The *oesophagus* is club-shaped, with the anterior portion forming the oesophageal funnel markedly dilated as the measurements will indicate. The maximum diameter is attained posteriorly, while the minimum diameter is approximately on a level with the ventral cervical groove.

The union of oesophagus with intestine is guarded by the usual three oesophageal valves.

The cells of the intestine were in sexually mature specimens heavily laden with dark pigment granules. This pigmentation, present throughout the length of the gut, was most pronounced in the anterior portion. It is the presence of this pigment which imparts the dirty grey to deep dark colour to the sexually mature adults. Posteriorly the lumen of the gut appears to have a cuticularized wall.

Male characters.

As the different measurements will be found in Table I, only the remaining characters will be considered. As regards colour, the males are slightly lighter than the females, which are undoubtedly more active feeders.

The *copulatory bursa* closely resembles that described for *O. apiostomum* and *O. xeri*. Variations in the distribution of rays were noticed and are figured. The prebursal papillae, situated about 400 μ from the caudal extremity, were prominent in some individuals, but less well defined in others owing to the longitudinal contraction with the resultant throwing of the cuticle into folds.

A prominent genital cone directed posteriorly and slightly ventrally arises by a broad base from the floor of the copulatory bursa. The *cloaca* communicates with the outside through a transverse ellipsoidal aperture situated subterminally on the dorsal face of the genital cone. Anterior to the anus the dorsal surface is indented by a transverse groove as figured by Goodey (1924) for *O. dentatum*.

The two lateral commissures of the cloacal aperture are each bounded laterally by a genital appendage, differing in shape with the angle from which they are viewed. In a ventral view they appear spherical with a ventrally directed appendage. When viewed laterally the spherical shape is replaced by a triangular one, with the apex of the triangle directed ventrally and guarding the protruding spicules. In the sexually mature males these appendages were best developed, indicating their relation to sexual activity.

The chitinized spicules are alated and of equal length. The alation stops within 66 μ of the free extremities, which terminate in fine turned-up points.

Female characters.

Posteriorly the female shows hardly any attenuation when examined with the unaided eye. On microscopical examination the maximum diameter was found to be reached at a point a little in front of the vulva. From this point the attenuation is rapid until the anus is reached. The tail is short, stout, blunt, and, in most of the specimens examined, tilted dorsally to be at right angles to the long axis of the body. The anus is situated at the posterior ventral angle of the thus formed foot. Near the tip of the tail there are the usually *caudal papillae*, somewhat ill-defined in most specimens.

The vulva is located on the anterior slope and near the summit of a well-marked elevation.

The vagina is of some length and is noteworthy in that it arises from the anterior border of the *pars ejectrix*. This appeared such an unusual phenomenon that two females were dissected with needles under a binocular microscope to verify this. The *pars ejectrix*, with concave anterior curvature and convex posterior curvature, occupies an oblique position in the body. The structure and general disposition of the remaining genitalia is typical as recorded for members of the genus. The posterior extremity in all adult females showed the usual brown cement material adherent.

Acknowledgements.

The writer wishes to express his indebtedness to his colleague, Mr. I. P. Marais, Veterinary Research Officer, for the material collected from the caecum and colon of springhares shot in the western Transvaal while investigating an outbreak of rabies amongst the small wild mammalia in that area.

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TABLE

The Measurements (in Millimetres) of the

Species	*O. brumpti.*	*O. apiostomum.*	*O. apiostomum.*
Hosts	African Negro .	*Macacus sinicus* *M. cynomolgus*	Man : W. Africa Monkeys : Africa
Habitat	Encysted in wall of caecum and colon	—	Great intestine.
Locality	Omo River....	—	Africa.........
Authors	Rail. and Henry (1905)	Rail. and Henry (1906)	Leiper (1911–1913)
Length: ♂	—	12·5	8
♀	8·5–10·2	14	10
Maximum width: ♂	—	0·4	—
♀	0·295–0·325	0·450	—
Length of oesophagus: ♂	—	0·550–0·60	0·400
♀	0·470–0·500		0·430
Maximum width of oesophagus: ♂	—	—	—
♀	—	—	—
Minimum width of oesophagus: ♂	—	—	—
♀	—	—	—
Maximum width of oesophagus posteriorly: ♂	—	0·165	—
♀	0·150		
Anterior extremity to cervical papilae: ♂	—	0·320	—
♀	0·320–0·370		
Anterior extremity to nerve ring: ♂	—	—	0·150
♀	—	—	
Anterior extremity to excretory pore: ♂	—	0·250–0·275	—
♀	—		
Height of buccal capsule: ♂	—	0·020	0·006
♀	0·015		
Internal width of buccal capsule, anteriorly: ♂	—	0·048	0·030
♀	0·035		
Internal width of breed capsule, posteriorly: ♂	—	0·065	0·030
♀	0·045		
Length of spicules	—	1·250–1·275	0·650
Vulva to caudal extremity	0·350–0·475	0·420	0·350
Vulva to anus	—	0·240	—
Anus to caudal extremity	0·170	0·180	0·200
Ova Length	—	—	0·063
Ova Width	—	—	0·027

I.

Various Species mentioned in the Text.

O. stephanostomum	*O. apiostomum.*	*O. xeri.*	*O. susannae.*	*O. susnanae.*
Man and monkeys, Africa	Orang-outan...	*Xerus setosus...*	*Pedetes caffra..*	*Pedetes caffra.*
Wall of the large intestine	—	Lumen and wall of caecum	Wall of caecum	Lumen of caecum and colon.
Africa.........	—	S. Africa......	E. Transvaal...	W. Transvaal.
Lane and Low (1922)	Ihle (1922).....	Ortlepp (1922).	Le Roux (1929)	Le Roux (1929).
6·5–11 8·5–12·5	10 –12·8 11·5–15	12–14 13–16·5	11 13–13·5	11·5–12 13 –17
0·3 —	0·425–0·565 0·530–0·655	0·380 0·510	0·374–0·382 0·425–0·442	0·426–0·474 0·442–0·600
— 0·5	0·615 0·685–0·720	0·600–0·700 0·650–0·825	0·595 0·578	0·537–0·585 0·577–0·585
— —	— —	— —	0·072–0·073 0·071–0·072	0·068–0·073 0·070–0·086
— —	— —	— —	0·052–0·054 0·055–0·059	0·050–0·059 0·059–0·066
—	0·170 0·165–0·170	— —	0·126–0·136 0·125	0·119–0·125 0·128–0·148
0·350	— —	— —	0·390–0·424 0·456–0·458	0·348–0·411 0·430–0·453
0·200	—	—	0·280–0·288	0·275–0·280
0·200	0·262–0·270 0·308	— —	0·271–0·280 0·270–0·272	0·253–0·270 0·260–0·275
—	0·021	—	Dorsal 0·013 Ventral 0·019	Dorsal 0·013 Ventral 0·017
—	Including walls ·060–0·070	— —	0·042 0·046	0·044–0·046 0·047–0·050
—	Including walls 0·078–0·082	— —	0·045 0·050	0·050–0·052 0·050–0·052
0·900–1·080	1·285–1·350	1·290	1·420	1·390–1·420
0·350–0·475	0·410–0·450	—	0·326–0·339	0·350–0·390
—	—	—	0·183–0·186	0·192–0·215
0·170–0·200	0·205–0·210	0·114–0·142	0·156–0·170	0·130–0·180
— —	— —	0·058 0·039	No ova in uterus	0·072 0·047

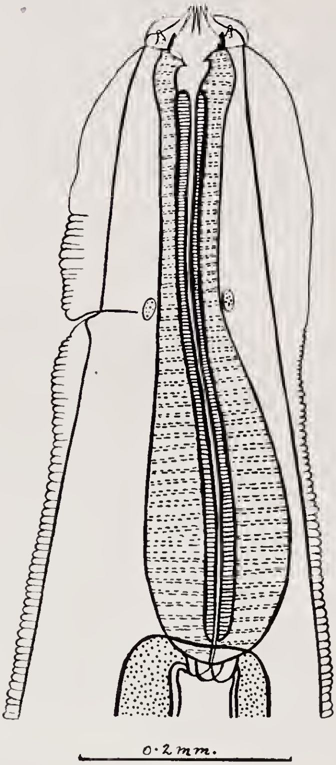


Fig 1.

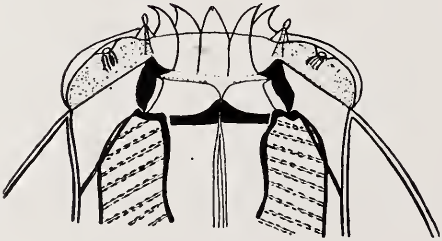


Fig 2.

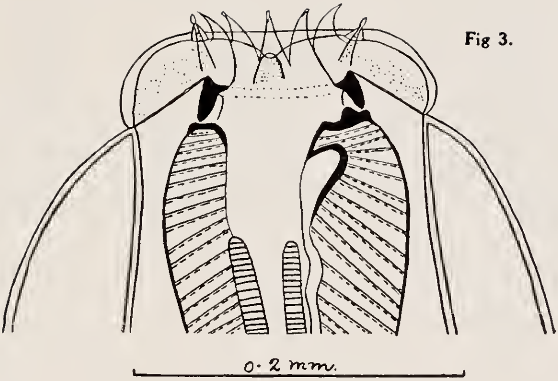


Fig 3.

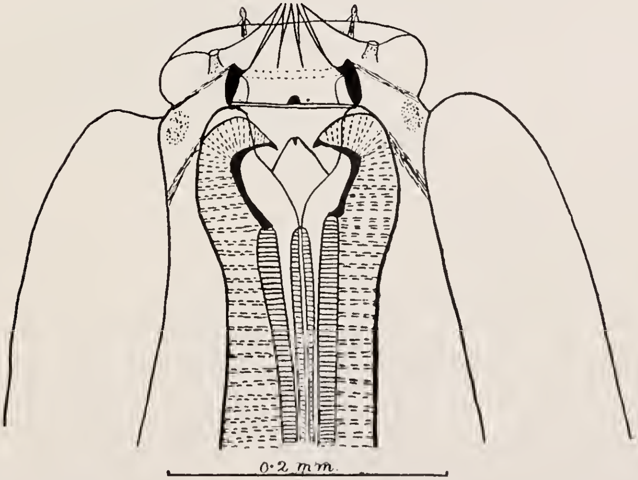
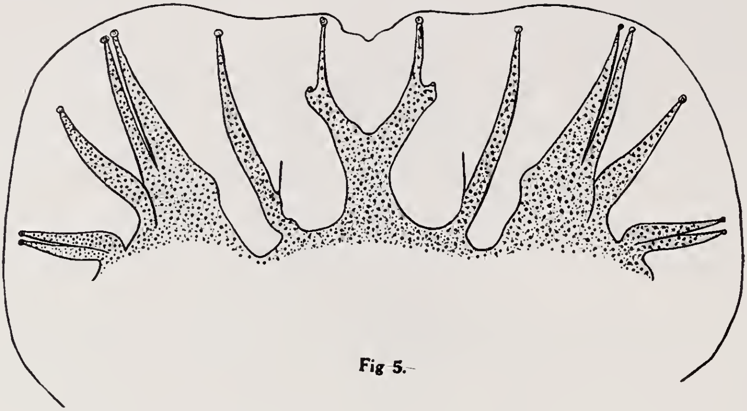


Fig 4.

Dorsal view, showing the dorsal gutter, the oesophageal teeth, etc.



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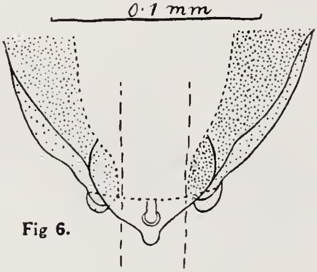

Fig 6.

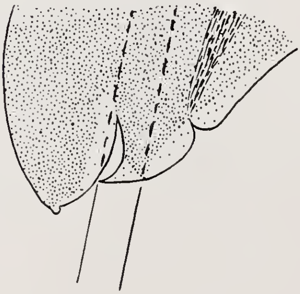

Fig 7.

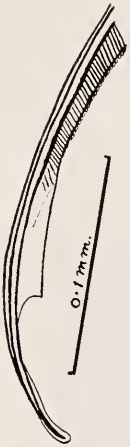

Fig 8.

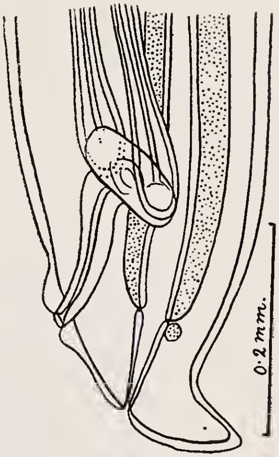

Fig 9.

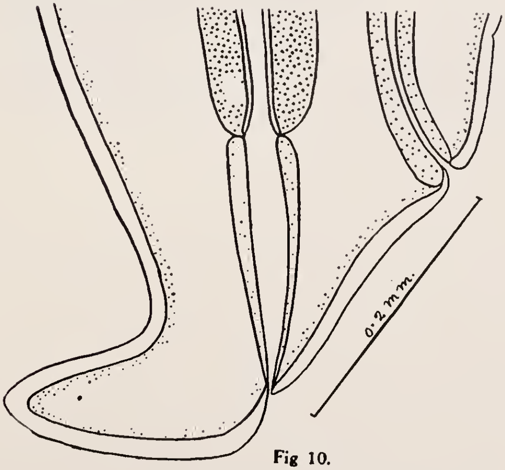

Fig 10.

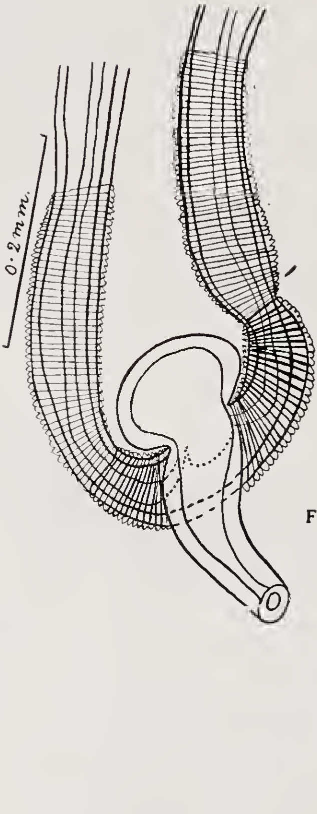

Fig 11.

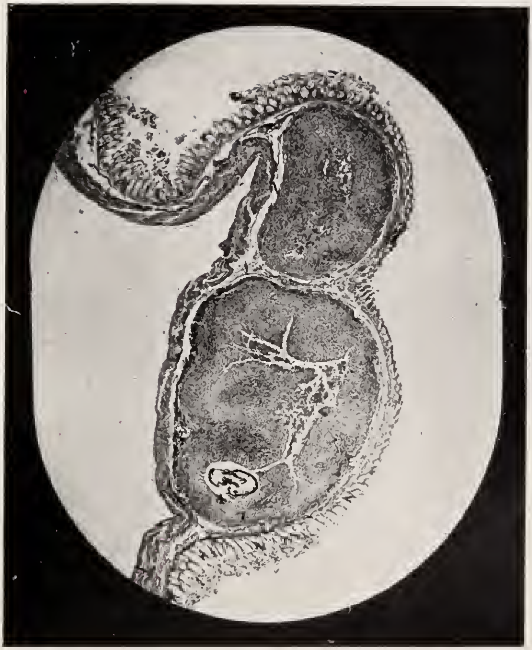

Fig. 12.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

On a Hookworm *(Agriostomum gorgonis,* sp. nov.) from the Blue Wildebeest *(Gorgon taurinus)* in the Transvaal.

By P. L. LE ROUX, B.Sc. (Edin.), M.R.C.V.S., Veterinary Research Officer, Onderstepoort.

INTRODUCTION.

THE type species, the only other member of the genus *Agriostomum* Railliet 1902, was described from the duodenum of a Zebu in Sumatra. Lane (1923) redescribed the species. He examined material from *Bos indicus* at Darjeeling, India. Ware (1925) having obtained specimens from the intestine of *Bos indicus* in the Nilgeri Hills, South India, repeated the description with some additions.

It seems desirable that the type specimens be re-examined to ascertain whether the bifid *dorsal tooth*, as figured by Ware (1928, Figure 1, A), is present. Railliet and Henry (1913) figure no such structure for *Agriostomum vryburgi*. Baylis and Daubney (1926) recognize Ware's " straight tooth-like organ " to be the dorsal gutter ending in a cone, bifid at the apex.

Agriostomum gorgonis, sp. nov.

This species was collected by Mr. G. A. H. Bedford from the *ileum* of blue wildebeest shot in the Waterberg District, northern Transvaal. These animals harboured also the specimens described as *Haemonchus bedfordi* Le Roux (1929).

The type specimens and the co-types are deposited in the helminthological collection at this Institute.

MORPHOLOGY OF " AGRIOSTOMUM GORGONIS," Sp. Nov.

The specimens preserved in glycerine alcohol are of a dirty white colour, and the body shows fine transverse striations. The portion of the gut immediately behind the oesophagus is very dark owing to the presence of dark granules in the intestinal cells and of dark-coloured ingesta in the lumen of the gut.

From about the oesophago-intestinal junction the males and the females taper towards both extremities. The female ends posteriorly in a fairly long tail acutely pointed. In the male the posterior extremity, owing to the folding in of the lateral lobes of the copulatory bursa, appears markedly attenuated. To the unaided eye the lateral width of the head approximates the maximum breadth of the body. Immediately posterior to the buccal capsule the body narrows to form a distinct neck.

The *oral aperture* faces dorsally and is bounded by the usual six *circum-oral papillae.*

The two *subventral cephalic papillae* are the most conspicuous and figure prominently in the hoodlike expansion of the cuticle extending anteriorly to the ventral margin of the mouth. The *medial* and the *sub-dorsal papillae* are opposite and lateral to the *lateral* and *dorso-lateral buccal teeth* respectively.

The oral aperture, somewhat hexagonal in outline, leads into a spacious buccal cavity with thick cuticularized wall, which has its anterior border armed with fourteen teeth and a cutting plate. These structures are arranged approximately as figured (Figs. 1 and 2).

The two *subventral teeth* are fused medially at the base to form the pair of ventral teeth described and figured for *Agriostomum vryburgi* Railliet (1902). The remaining twelve teeth are grouped into six pairs; two dorso-lateral, two lateral, and two ventro-lateral. Each pair consists of a well-developed external tooth and a shorter, but stout, inner tooth, the latter being partly hidden by the former. One or more of these buccal teeth may occasionally be found with the tip or tips eroded as recorded by Ware (1925) for *A. vryburgi* and by Schwartz (1927) for *Ancylostoma pluridentatum.*

Dorsally there is a short but broad-based cutting plate with semicircular convex cutting edge. The functions of this structure and of the buccal teeth are apparent. That this nematode is an active bloodsucker like the other members of the subfamily cannot be disputed.

The *dorsal oesophageal gland*, with duct penetrating the buccal capsule dorsally, opens into the buccal cavity at the summit of a slight elevation situated behind the dorsal cutting plate (Figure 4).

Anteriorly the medial surface of the mouth capsule carries a projecting structure (Figure 5) suggesting an internal leaf crown.

The cuticularized buccal capsule articulates posteriorly with a circular cuticular ring, surmounting the anterior edge of the oesophagus.

The spacious buccal cavity, with ventral wall much higher than the dorsal one, leads into a well-developed *oesophageal funnel.*

The *oesophagus* is club-shaped, muscular, and anteriorly has its lumen dilated to form the voluminous oesophageal funnel. The oesophago-intestinal union is guarded by the usual valves, well-developed in this species.

The lumen of the intestine is packed with darkish coloured material, probably partly digested blood and epithelial cells. The intestinal cells are packed with a blackish pigment.

The *cervical papillae*, situated about the level of the nerve ganglion, are inconspicuous and may readily escape notice.

The *cervical glands* have the *excretory pore,* not situated in a *cervical cleft* as recorded for the type species. The excretory pore is approximately on a level with the cervical papillae.

MALE CHARACTERS.

The *genital bursa* in preserved specimens has the lateral lobes folded in. The arrangement of the rays agrees well with that described for *A. vryburgi*. In the majority of individuals the *dorsal* and the *externo-dorsal rays* arise from a stout and comparatively long common trunk. Each *dorsal ray* terminates in two branches, a slender inner branch and a stout lateral branch. The latter is occasionally furnished with a medial accessory offshoot. The *postero-lateral*, the *medio-lateral*, and the *externo-lateral* have a common trunk. The first to branch off is the *externo-lateral*. The common trunk to the *postero-lateral* and the *medio-lateral* is short and the rays lying close together are stout. The *ventral rays*, closely applied, terminate near the edge of the bursa. Variations met with are figured. The *prebursal papillae* are slender and do not project beyond the cuticle in the specimens examined.

The *genital cone* is of the typical ancylostome type, and the spicules are provided with transversely striated alae (Fig. 13).

The *accessory piece*, which in *A. vryburgi* is recorded by Ware (1925) as clavicular in a lateral view and heart-shaped in a dorsal view, is part of a rather ill-defined *telamon*. In whole specimens cleared in lacto-phenol the exact limits of this structure could not be ascertained with certainty. The *telamon* with the spicules attached was dissected out, but even then its outlines were obscured by the muscles adhering to it. When viewed dorsally its anterior portion carries a structure heart-shaped in outline (Fig. 12) and measuring approximately 70 μ × 48 μ. This structure is undoubtedly analogous to the *accessory piece* figured for the type of the genus. According to Ware's drawing (Fig. 1 B) it measures 114 μ × 85 μ. Below this heart-shaped structure referred to above is another similarly shaped part, measuring 42 μ × 32μ. This is apparently a portion of the first mentioned and is situated between the two spicules. The whole structure separates the spicules and secures them in position and forces them to assume a certain direction as they emerge beyond the *telamon*. The alae with their free borders medially directed (Fig. 12) and overlapping the dorsal surface of the first-mentioned heart-shaped structure overlap and envelop each other, and so doing secures the free extremities of the spicules firmly together. Laterally the main body of the *telamon* carries two posteriorly directed wings which secure the spicules laterally. A short distance posterior to their termination the wall of the spicular canal appears again to be slightly chitinized.

Female Characters.

The female is larger than the male, with the posterior extremity ending in a fine tail.

The *genitalia* are of the *oesophagostomum* type. The *vagina* is short and the *vulva*, semilunar in shape, opens on a well-marked ventral prominence (Figure 3).

The *caudal papillae* are readily seen when the worm is rotated. From these the body rapidly narrows, to end in a long thin caudal appendage (Figure 3).

Identity of the Species.

That this parasite is a species distinct from *A. vryburgi* Railliet (1902), is proved by (*a*) the shape and the dimensions of the buccal capsule, (*b*) the size of the oesophageal funnel, (*c*) the number of buccal teeth, and (*d*) the absence of a *cervical groove*.

Lane (1923) points out that *A. vryburgi* forms an interesting link between the Ancylostomidae and the Oesophagostomidae. He proposes for it the subfamily *Agriostominae,* which is included in the family Oesophagostomidae. This suggestion for its inclusion in the family Oesophagostomidae cannot be supported, and it should be retained in the *Ancylostominae* as suggested by Ihle (1922) and by Baylis and Daubney (1926).

TABLE I.

The Measurements (in millimetres) of Males and Females of Agriostomum gorgonis, *sp. nov.*

	Males.	Females.
Length of worm............................	9–10	11–13
Lateral width of head......................	0·286–0·340	0·320–0·360
Maximum width of body....................	0·312–0·360	0·340–0·370
Width of body in front of copulatory bursa...	0·231–0·280	—
Length of oesophagus.......................	1·400–1·500	1·440–1·500
Maximum diameter of oesophagus anteriorly...	0·234	0·243
Maximum diameter of oesophagus posteriorly..	0·240–0·280	0·250–0·280
Minimum diameter of oesophagus............	0·140–0·160	0·140–0·180
Lateral width oral aperture..................	0·200–0·220	0·230–0·240
Dorso-ventral diameter of buccal cavity.......	0·156–0·172	0·154–0·176
Lateral diameter of buccal cavity............	0·187–0·190	0·180–0·200
Height of dorsal wall of buccal cavity........	0·020–0·025	0·020–0·025
Height of ventral wall of buccal cavity.......	0·240–0·260	0·260–0·280
Lateral width of oesophageal funnel..........	0·180–0·210	0·200–0·230
Height of dorsal wall of oesophageal funnel...	0·125–0·156	0·130–0·165
Height of ventral wall of oesophageal funnel..	0·186	0·189
Length of spicules..........................	1·00 –1·20	—
Anterior extremity to excretory pore..........	0·700–0·840	0·780–0·844
Anterior extremity to cervical papillae........	0·800–0·900	0·900–0·950
Vulva to anus..............................	—	0·240–0·300
Anus to posterior extremity..................	—	0·240.0·310

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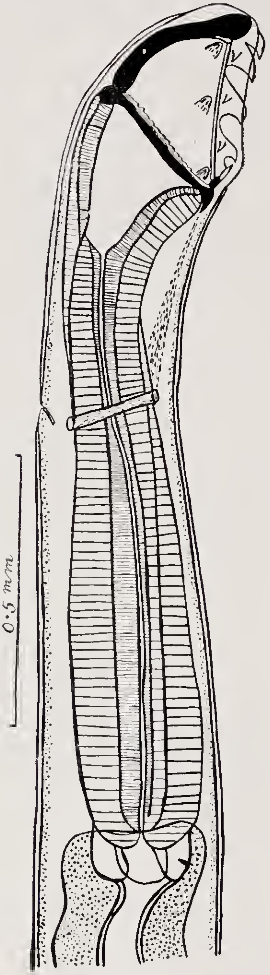


Fig 1.

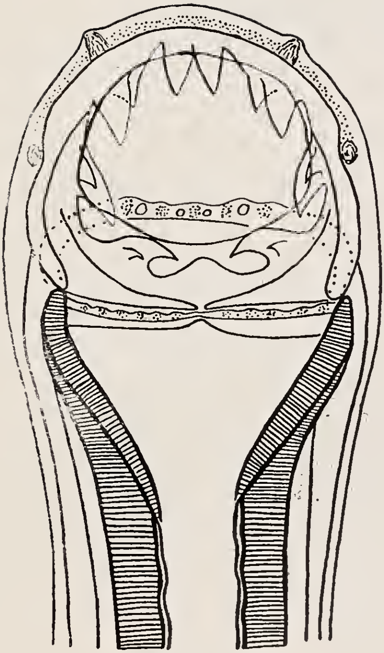


Fig 2.

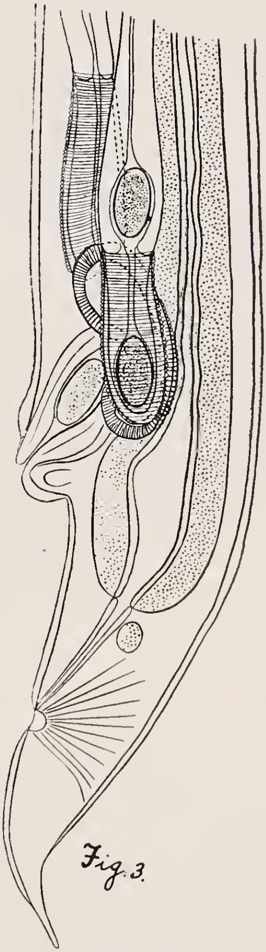


Fig. 3.

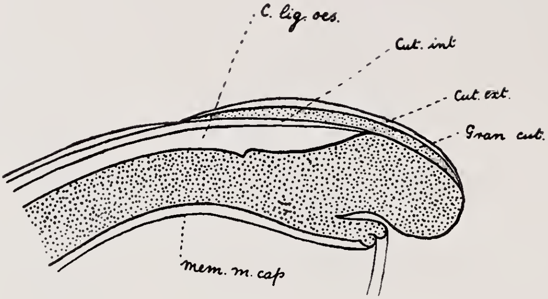


Fig 4.

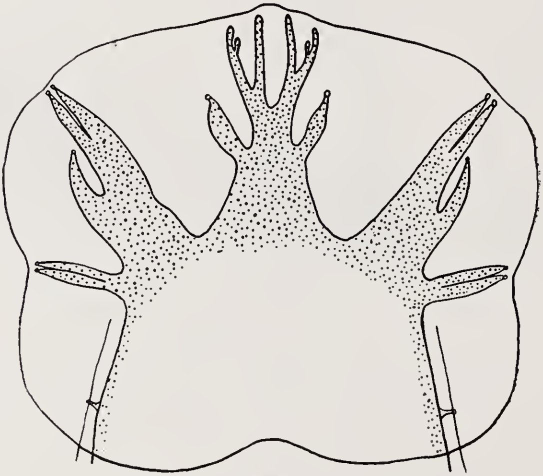


Fig 5.

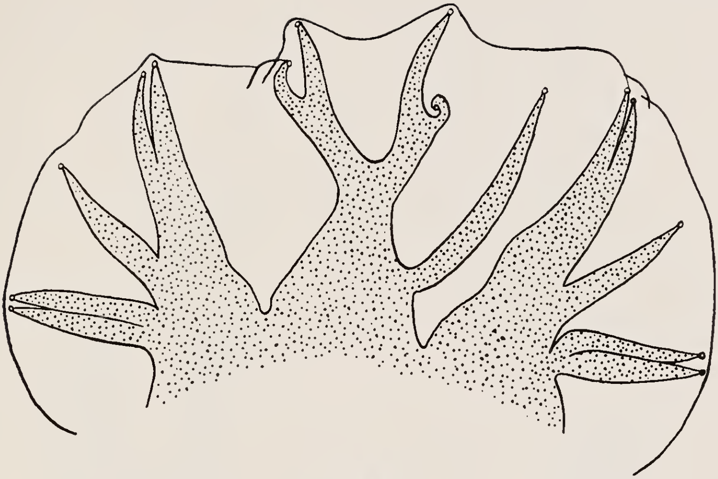


Fig 6.

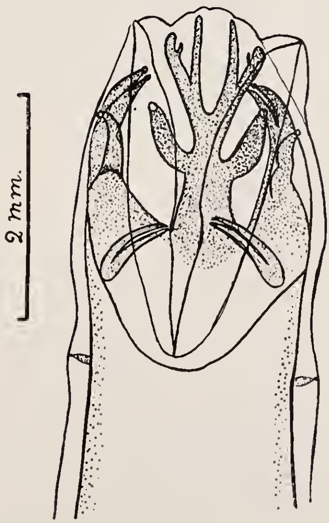


Fig 7.

Fig 8.

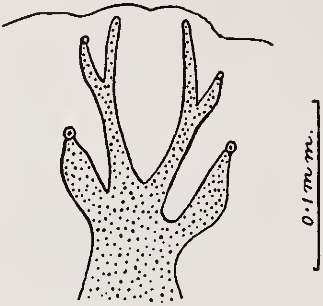
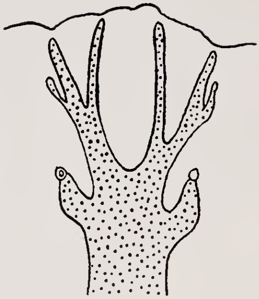


Fig 9.



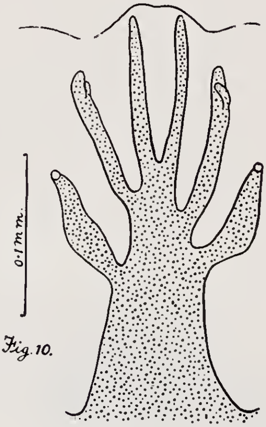


Fig. 10.

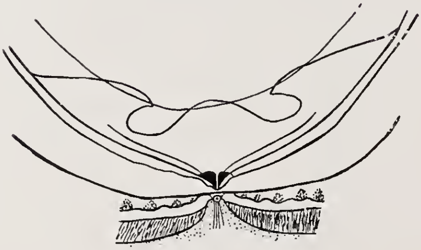


Fig. 11.

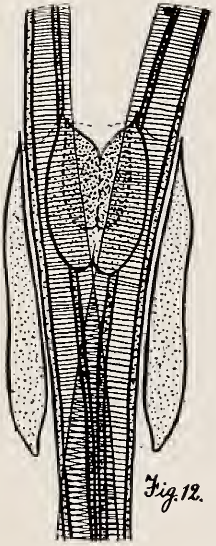


Fig. 12.

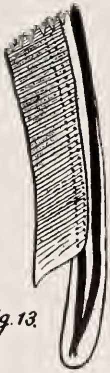


Fig. 13.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

Notes on some South African Ticks, with Descriptions of Three New Species.

By G. A. H. BEDFORD, Research Officer, Onderstepoort.

Genus Ornithodorus C. L. Koch.

Ornithodorus pérengueyi Bedford and Hewitt.

Ornithodorus pérengueyi Bedford and Hewitt, *S. Afr. Journ. Nat. Hist.*, V, i, pp. 259, 260, Pl. 19, f. 1-3 (1925).

A number of adults and nymphs were found in the nests of *Petrochelidon spilodera* Sund. (South African cliff swallow) at Onderstepoort in March and April, 1927 (coll. G. A. H. B.). This species was described from adults and one nymph collected in a native church at Nqamakwe, C.P. The host was not recorded.

Genus Ixodes Latreille.

Ixodes elongatus nov. sp.

(Plate I, figures 1, 2.)

Female (unengorged): *Scutum* dark reddish-brown in colour, much longer than broad (1.07 mm. × 0.64 mm.), widest in the middle; cervical grooves short, defined by short, narrow depressions, extending backwards from behind the anterior margin to beyond middle of scutum; lateral grooves well developed; punctations equal, fairly large and deep, with a white hair arising from the majority of them, the hairs being longer in front and on each side beneath the antero-lateral angles. Body 1.78 × 1.05 mm. *Dorsum* paler than scutum, with numerous pale hairs; grooves absent. *Venter* with similar hairs; vulva situated in a line between coxae iii and iv; anal groove horseshoe shaped. Spiracles large, circular. *Capitulum* with the dorsal base slightly broader than long, 0.17 mm. long × 0.24 mm. broad, narrower in front than behind; porose areas well separated. Palpi very slightly longer than hypostome, narrow, second article less than twice the length of third. Hypostome 3/3 in front, 2/2 behind, the posterior inner teeth shorter and blunter than the outer teeth. *Legs* long and slender; coxae broad, flat; coxae i with a medium-sized spur at the postero-external angle and with a short one internally; coxae ii, iii, and iv with a spur at the postero-external angle, those on coxae ii and iii minute.

Male: unknown.

Described from a single female taken off *Mastomys coucha* A. Smith (multimammate mouse) at Onderstepoort on the 6th February, 1929 (coll. P. L. le Roux).

This species appears to be closely allied to *I. nairobiensis* Nuttall, described from a single female taken off a dog at Nairobi, Kenya Colony, and to *Ixodes acuminatus* Neumann, described from two females taken off a rat in Italy. From both these species it can be distinguished, *inter alia*, by the shape of the anal grooves and the spurs on the coxae. *Ixodes vespertilionis* Koch, found on bats in Europe, N. Africa, and Australia, is probably more closely related to these three species than to any other known species of *Ixodes*. In the female of this species the scutum resembles that of *elongatus* in shape, but differs from *elongatus*, *nairobiensis*, and *acuminatus* in the shape of both the base of the capitulum and the anal groove; also the legs are longer and there are no spurs on the coxae.

IXODES RASUS Neumann.

Ixodes rasus Neumann, *Mem. Soc. Zool. France*, xii, p. 137, f. 12-14 (1899).

Ixodes rasus Neumann, *ibid.*, xiv., p. 285 (1901).

Ixodes rasus Neumann, *Herausg. v. d. Königl. Schwed. Akad. d. Wiss. Upsala*, p. 18 (1907).

Ixodes rasus Neumann, *Das Tierreich. Ixod.*, p. 26 (1911).

Ixodes rasus Nuttall and Warburton, *Mon. Ixod.*, pp. 228-230, f. 224-226 (1911).

One female and three nymphs taken off *Procavia capensis natalensis* Roberts (Natal dassie) at Mtabamhlope, Estcourt District, Natal (collected by Mr. P. Barnes and presented by Mr. Lawrence Hill). I am indebted to Mr. Warburton for kindly comparing these specimens with specimens in the Cambridge collection. Mr. Warburton informs me that this species is very variable, and that the specimens may prove to be a distinct variety.

Neumann has recorded *rasus* from *Procavia* sp., *Herpestes ichneumon*, *Cephalopus leucogaster*, and *Canis familiaris*, and gives the following localities: Congo, Cameroons, Togo, and Tanganyika Territory. Nuttall and Warburton have recorded specimens taken off cattle in Uganda, and from man, dog, and leopard in Ashanti.

Genus HAEMAPHYSALIS C. L. Koch.

HAEMAPHYSALIS COOLEYI nov. sp.

(Plate II, figure 3 A to D.)

MALE: *Scutum* reddish-brown in colour, oval, 1.88 × 1.3 mm., fairly narrow in front and broad behind, with numerous small punctations which are practically equal in size; cervical grooves short, curved inwards, moderately deep in front; lateral grooves well marked, extending backwards to the first festoon. *Capitulum* wider than long, 0.11 × 0.26 mm., the base with lateral margins winged, posterior margin straight without cornua. Palpi with article ii very broad and salient laterally, longer than article iii, article iii with a broad backward projecting process on the venter. Hypostome 4/4 with equal teeth.

Legs: coxae i and iv with small pointed spur at the internal angle; coxae ii and iii with a slight blunt spur at the internal angle; trochanter i with a strong spur on the dorsum.

Venter: reddish-brown with numerous small punctations, in the middle of each arises a minute white hair; spiracles ovate, the pointed end dorsal.

FEMALE (unengorged): *Scutum* reddish-brown in colour, about as wide as long, 0.87 mm. long × 0.86 mm. wide; cervical grooves short, curved; punctations smallish, equal in size and evenly distributed. *Body* same colour as scutum, with numerous punctations on both dorsum and venter; lateral grooves well marked, extending backwards to end of second festoon. *Capitulum* wider than long, 0.43 mm. long × 0.16 mm. wide, the base with lateral margins winged, posterior margin slightly concave without cornua; porose areas elongated-oval, well separated. Hypostome 4/4, with equal teeth. Palpi and legs as in the male.

NYMPH (unengorged) reddish-brown in colour. Scutum slightly broader than long, 0.41 × 0.48 mm., with a few small punctations, arising from the middle of each is a minute white hair. Marginal grooves well developed, extending back to the end of the first festoon. Capitulum wider than long, 0.08 × 0.21 mm., the base with lateral margins rounded, posterior margin slightly concave. The palpi differ slightly from those of the female, article ii being shorter behind the lateral angle and resembling more closely that of *H. hoodi* Warb. and Nutt. in shape, and on the venter of the second article there is a backward projecting spine. Legs as in the female, except that the spurs on coxae i-iii are slightly more developed, those on ii and iii being more pointed; coxae iv without a spur.

Described from three males, one female, and two nymphs taken off *Procavia capensis coombsi* Roberts (Transvaal dassie) near Onderstepoort on the 22nd August, 1928 (coll. Prof. R. A. Cooley and G. A. H. Bedford), also several engorged nymphs taken off *Pedetes caffer* Pall. (springhare) at Pienaars River, Tarnsvaal, on the 1st October, 1928 (coll. Prof. R. A. Cooley).

Holotype: a male from *P. capensis coombsi*.

This species is closely allied to *H. hoodi* Warburton and Nuttall (found on birds in various parts of Africa), from which it can be readily recognized by the shape of the palpi, and the cervical grooves of the male are shorter than those of *H. hoodi*. A variety of *hoodi* has been described from specimens taken off *Procavia manningi* in Nyasaland, but it appears to be quite distinct from *cooleyi*.

Genus RHIPICEPHALUS Koch.

RHIPICEPHALUS PUNCTATUS nov. sp.

(Plate III, figure 4 B and D, and figure 5 B.)

MALE: *Scutum* oval, more than twice as wide posteriorly as in front, length 2.26 mm., width 1.5 mm., reddish-brown in colour, shining, with very few punctations, those on the posterior half being particularly large and deep; arising from each punctation there is a minute pale hair; eyes flat, yellowish; cervical grooves short; lateral grooves extending back to first festoon; festoons well developed. *Capitulum* wider than long, length 0.43 mm., width 0.5 mm., lateral margins of base with prominent angle, posterior margin very slightly concave without pronounced cornua. Palpi longer than wide, article ii longer than article iii.

Legs reddish-brown in colour, coxae i with internal spurs pointed and about as wide as the external spurs.

Venter reddish-brown with a few punctations, each with a small pale hair in the middle; adanal shields with the outer and posterior margins convex, inner margins concave; accessory shields normal.

FEMALE (slightly engorged): *Scutum* slightly wider than long, length 1.26 mm., width 1.36 mm., dark reddish-brown, shining, with a few large punctations on the median area and on each side in front; eyes flat, yellowish; cervical grooves very short, curved inwards; lateral grooves entirely absent. *Capitulum* 0.66 mm. long, 0.75 mm. wide; base without pronounced cornua; porose areas rounded.

Body same colour as scutum; marginal grooves indistinct, median and postero-lateral grooves narrow; festoons distinct.

Described from seven males and a single female taken off a *Procavia capensis coombsi* Roberts (Transvaal dassie) near Onderstepoort on the 22nd August, 1928 (Coll. Prof. R. A. Cooley and G. A. H. Bedford); also two males and two females received from Dr. Schmidt taken off a dassie at Omaruru, South-West Africa.

Holotype: a male from *P. capensis coombsi*.

This new species resembles *R. simus* Koch, and for comparison we also give figures of that species drawn to the same scale, these specimens being of normal size. The males are smaller than those of *simus* and reddish-brown instead of dark brown to black. In *simus* the scutum varies from about 2.6 × 1.85 mm. to 3.9 × 2.8 mm., the former being abnormally small. The punctations on the scutum of the male are very much larger than those of *simus*. In the latter species they are variable, there usually being a number of very small punctations present in addition to the large ones, which are invariably arranged as shown in the figure. The internal spurs of coxae i are much wider than the external spurs in *simus* (compare figure 4 C and D). In *simus* the cornua of the basis capituli are very distinct in both sexes, whereas in *punctatus* they are inconspicuous. The females can also be distinguished from *simus* by the shape of the scutum, the cervical grooves being short instead of long, the absence of lateral grooves, and the punctations are considerably less numerous. In *simus* the punctations in the median area between the lateral grooves are usually small with a large one dotted about here and there, not as in figure 5 A, in which they are all large.

We have no hesitation in regarding *punctatus* as a good species for the following reasons: Firstly, the specimens of *R. simus* drawn for comparison were taken off cattle on the same farm as the specimens of *punctatus*. We have examined a large number of *simus* collected on cattle and dogs in this locality, but not one of them resembles this new species. Secondly, typical *punctatus* have also been found on a dassie in South-West Africa; and, lastly, the females of the species of *Rhipicephalus* are as a rule very difficult to distinguish, whereas the females of *punctatus* are very distinct and easy to identify.

PLATE I.

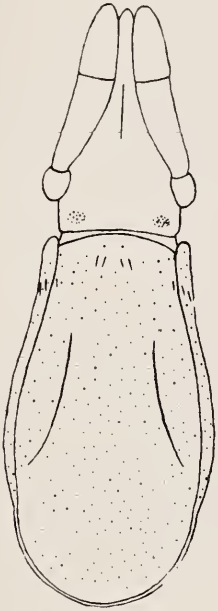


FIG. 1.

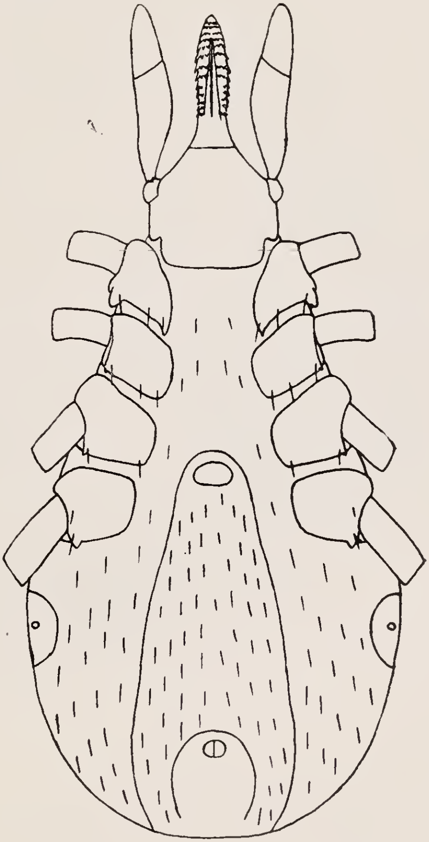


FIG. 2.

FIG. 1. *Ixodes elongatus* nov. sp., capitulum and scutum of female.

FIG. 2. *Ixodes elongatus* nov. sp., venter of female.
A. Posterior teeth of hypostome.

G.A.H. B. del.

PLATE II.

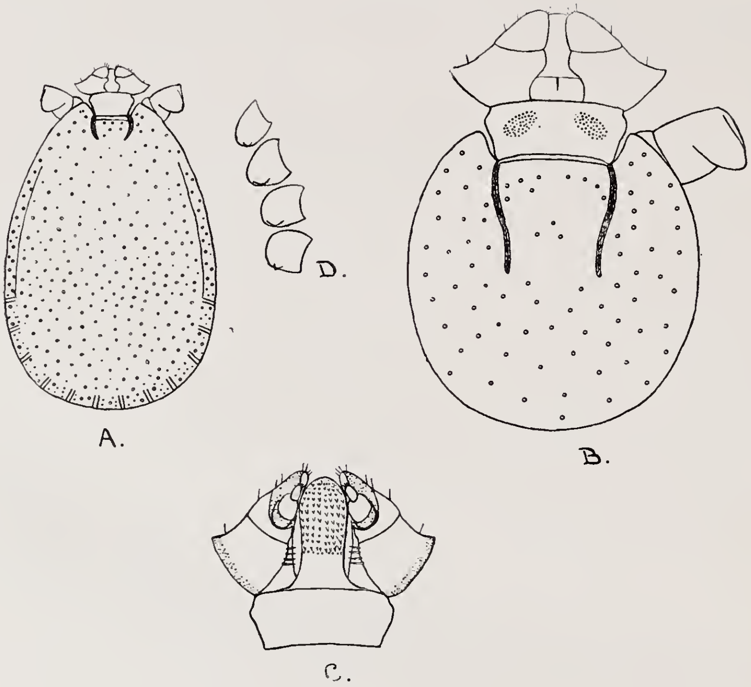


FIG. 3.

Haemaphysalis cooleyi nov. sp. A. Dorsum of male; B. Capitulum and scutum of female; C. Capitulum in ventral aspect of female; D. Coxae of male.

G.A.H. B. del.

PLATE III.

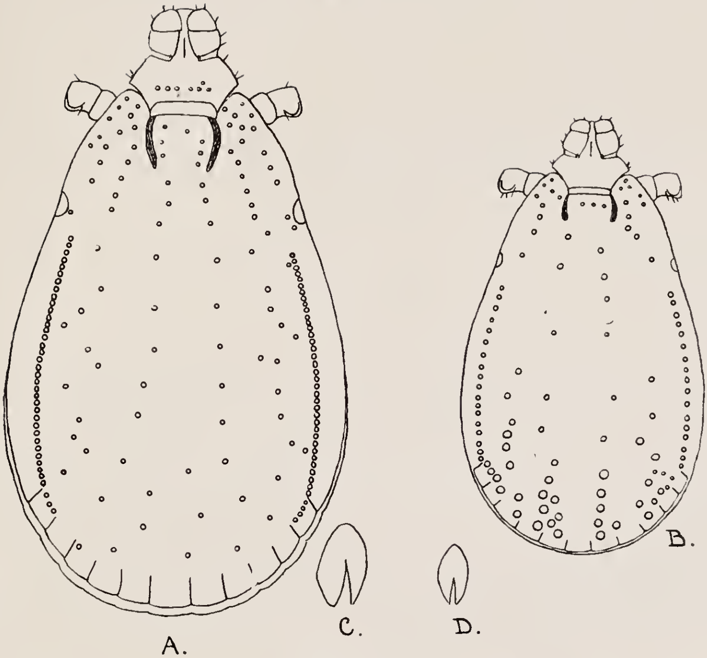


FIG. 4.

A. and C. Dorsum and coxa i of male of *Rhipicephalus simus* Koch.
B. and D. Dorsum and coxa i of male of *Rhipicephalus punctatus* nov. sp.

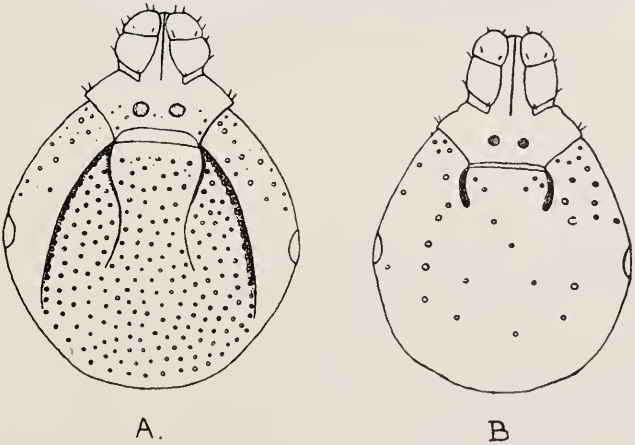


FIG. 5.

A. Capitulum and scutum of female of *Rhipicephalus simus* Koch.
B. Capitulum and scutum of female of *Rhipicephalus punctatus* nov. sp.

G.A.H. B. del.

15th Annual Report of the Director of Veterinary Services,
Union of South Africa. October, 1929.

Anoplura *(Siphunculata and Mallophaga)* from South African Hosts.

By G. A. H. BEDFORD, Division of Veterinary Services,
Onderstepoort.

The present paper is based mainly on a collection of Anoplura belonging to the South African Museum and submitted to the author by Dr. R. F. Lawrence for identification. The majority of the species were collected by Dr. Lawrence in Ovamboland, South-West Africa, in 1922-23, and a few by Mr. White at Capetown. In addition to these, there are also described or recorded a few species in the collections of the Division of Veterinary Research and of Mr. Lawrence Hill, of Pietermaritzburg.

We wish to express our thanks to Mr. C. G. Walker for the great care he has taken in drawing the figures reproduced in this paper, with the exception of figures 2 to 5 and 20 and 21, which were drawn by the author.

Sub-order SIPHUNCULATA.

Genus Lemurphthirus Bedford.

Lemurphthirus Bedford, *Parasit.*, XIX, ii, p. 263 (1927).

1. Lemurphthirus galagus Bedford.

L. galagus Bedford, *Parasit.*, XIX, ii, pp. 263-264 (1927).
(Figure 1.)

Two males (one slightly immature) taken off a lemur, *Galago moholi*, in South-West Africa. This species was described from a single female taken off the same host in the Transvaal.

Description of Male.—Head as in the female. Second joint of antennae shorter than that of female, with a short spine on the inner margin; third joint with two slightly stronger spines, one at the distal post-axial angle and the other behind it.

Thorax and *legs* as in the female.

Abdomen elongated; tergites i to vii each with a very narrow median, transverse dark band and four long bristles, with a short spine between the outer pairs and one long bristle inside each spiracle. Tergite viii with two long bristles in the middle and two long spiracular bristles on each side. Apical tergite with a small plate on each side on the posterior margin with two short hairs. First sternite with a longish bristle on each side of the meson; second to eighth sternites with similar bands and bristles as on the dorsum, the spines between the bristles being slightly smaller than those on the dorsum. On the second sternite the band is broad and there are an additional two bristles above the transverse row. Pleurites as in the female, only present on the second segment. Spiracles present on the third to eighth segments. Male genitalia as in Fig. 1.

Measurements.

	Length.	Width.
Head	0.25	0.25
Thorax	0.28	0.26
Abdomen	0.68	0.41
Total	1.21 mm.	

Genus LINOGNATHUS Enderlein.

Linognathus Enderl., *Zool. Anz.*, XXIX, p. 194 (1905).

1. LINOGNATHUS VITULI (Linnaeus).

One immature specimen found on a fly. *Musca lasiophthalma*, at Camps Bay, Capetown, in May, 1928.

2. LINOGNATHUS GORGONUS nov. nom.

L. ferrisi Bedford, *Trans. Roy. Soc. S. Afr.*, XIV, iv, pp. 351, 352, Fig. 5, 6 (1927), *nec* Fahrenholz, 1919.

As Dr. Maurice C. Hall has very kindly pointed out to me, my *L. ferrisi* is preoccupied by *L. ferrisi* Fahrenholz, 1919. I now, therefore, propose the name *gorgonus* for the species parasitic upon *Gorgon taurinus* (blue wildebeest).

Genus EULINOGNATHUS Cummings.

Eulinognathus Cummings, *Ann. Mag. Nat. Hist.* (viii), xvii, p. 90 (1916).

1. EULINOGNATHUS DENTICULATUS Cummings.

E. denticulatus Cummings, *Ann. Mag. Nat. Hist.* (viii), xvii, pp. 90-94, f. 1 (1916).

Three females taken off *Pedetes caffer* Pall. (springhare).

Genus LINOGNATHOIDES Cummings.

Linognathoides Cummings, *Bull. Ent. Res.*, V, p. 159 (1914).

Ferris (*Stanford Univ. Publ.*, II, iv, p. 237, 1923) has recently sunk this genus as a synonym of *Neohaematopinus* Mjöberg, but as the following species was described as a *Linognathoides* and its generic position is doubtful I am retaining it here.

1. LINOGNATHOIDES FAUREI Bedford.

L. faurei Bedford, *Seventh and Eighth Repts. Dir. Vet. Res.*, Un. S. Afr., pp. 710-712, Pl. 1, f. 2; Pl. 7, f. 3 (1920).

Three females taken off *Geosciurus capensis* Kerr (ground-squirrel).

Genus POLYPLAX Enderlein.

Polyplax Enderlein, *Zool. Anz.*, XXVIII, pp. 139, 142, 223 (1904).

Polyplax (Enderl.) Ferris, *Stanf. Univ. Publ., Biol. Sci.*, II, iv, p. 183 (1923).

1. POLYPLAX SPINULOSA (Burmeister).

Pediculus spinulosus Burm., "Rhynchota," *Gen. Ins.*, p. 8 (1839).

Polyplax spinulosa (Burm.) Ferris, *Stanf. Univ. Publ., Biol. Sci.*, II, iv, pp. 187-191, f. 119, 120 (1923).

Females and males collected by the writer off a rat, *Thallomys moggi* Roberts, along with specimens of *Hoplopleura affinis* (Burm.) at Onderstepoort in March, 1927.

2. POLYPLAX PRAOMYDIS nov. sp.
(Fig. 2-5.)

FEMALE.—Total length, 1.74 mm. Length of head 0.24 mm.; thorax (along middle line), 0.15 mm.; abdomen, 1.35 mm. Breadth of head, 0.15 mm.; thorax, 0.25 mm.; abdomen (4th and 5th segments), 0.46 mm.

Head (Fig. 2) longer than wide, acutely rounded in front of the antennae. On the dorsum there are two small hairs in front, and four a short distance behind the anterior margin, the lateral ones being close to the base of the antennae. Behind the antennae there is a transverse suture, and three minute hairs on each side in front of the suture. Lateral margins of hind-head almost parallel with one minute hair. At each latero-posterior angle there is a long bristle and two short ones above it, and on each side of the meson in the occipital area there is a minute hair. On the venter there is a row of four short hairs in front of the antennae, and a longer pair between the antennae. Antennae set a short distance back from the anterior margin; first segment of normal size with a strong short bristle on the dorsum.

Thorax shorter than the head, with the lateral margins convex, posterior margin concave. On the posterior margin of the mesothorax there is a very short spine just within the spiracle, and a much longer bristle slightly nearer the meson. Sternal plate as in Fig. 4.

Abdomen elongated, widest at the fourth and fifth segments. Tergite i with two hairs; tergite ii with one row of four hairs; tergite iii with one plate and one row of six hairs; tergites iv-vi each with two rows of seven to eight hairs, tergite vii with seven to eight hairs in the first row and six in the second. Sternite ii with two rows of hairs, three to five in the first row and four in the second; sternite iii with four to five hairs in the first row and six in the second; sternites iv-vi each with seven hairs in the first row and six in the second; sternite vii with five to seven hairs in the first row and six in the second. Gonopods normal, each with two short hairs and one long bristle.

Pleural Plates.—Those of the second segment divided, each with a short seta; plates of the third to sixth segments triangular, each with two short setae, the outer setae on the third segment are slightly shorter than the inner, and on the fourth to sixth segments the outer setae are slightly longer than the inner ones; plates on the seventh and eighth segments small, each with the usual two long setae.

MALE.—Total length, 1.16 mm. Length of head, 0.23 mm.; thorax, 0.13 mm.; abdomen, 0.8 mm. Breadth of head, 0.15 mm.; thorax, 0.21 mm.; abdomen, 0.36 mm.

Tergite i as in the female; tergite ii with one row of six hairs, the one on each side of the median pair being shorter than the others; tergites iii to vii each with one plate and one row of eight to nine hairs, those on each side of the middle pair being shorter than the rest; tergite viii with one row of four hairs. Sternite ii with two rows of hairs, three to four in the first row and four in the second; sternite iii with two plates and two rows of hairs, the anterior with three to four hairs and the posterior with four to six; sternites iv to vii each with one plate and one row of four hairs; sternite viii with two admedian hairs.

Genitalia as in Fig. 5; the basal plate is narrow, the parameres are short and the pseudopenis elongated.

Described from several females and males taken from a *Praomys namaquensis monticularis* Jameson (family Muridae) at Onderstepoort by the writer on the 1st August, 1927.

Holotype: a female.

This new species belongs to the *spinulosa* group, and can be easily distinguished from the other members by the pleural plates and shape of the head, which closely resembles that of *P. otomydis* Cumms.

3. POLYPLAX WATERSTONI Bedford.

P. waterstoni Bedford, *Sixth and Seventh Repts. Dir. Vet. Res.*, Un. S. Afr., pp. 715-6, f. 1, 2, 4, 5 (1919).

P. waterstoni (Bedford) Ferris, *Stanf. Univ. Publ.*, II, iv, p. 193-6, f. 121, 122 (1923).

Several females and males taken off *Mastomys coucha* A. Smith (multimammate mouse) by the writer at Onderstepoort and Sycamore in the Transvaal in 1927.

4. POLYPLAX CUMMINGSI Ferris.

P. cummingsi Ferris, *Ann. Durban Mus.*, I, pp. 240-3, f. 25, 26a (1916).

P. cummingsi Ferris, *Stanf. Univ. Publ.*, II, iv, pp. 213-5, f. 136, 137 (1923).

Several females and males collected by the writer off *Aethomys chrysophilus* de Wint. (African rat) at Sycamore, eastern Transvaal, on the 27th October, 1927.

Genus HOPLOPLEURA Enderlein.

Hoplopleura Enderlein, *Zool. Anz.* XXVIII, p. 221-223 (1904).
Hoplopleura (Enderl.) Ferris, *Stanf, Univ. Publ.*, *Biol. Sci.*, II, ii, p. 59 (1921).

1. HOPLOPLEURA AFFINIS (Burmeister).

Pediculus affinis Burm., "Rhynchota," *Gen. Ins.*, p. 10 (1839).
Hoplopleura affinis (Burm.) Ferris, *Stanf. Univ. Publ.*, *Biol. Sc.*, II, ii, pp. 75-79, f. 42, 43 (1921).

Males and females taken by the writer off a rat, *Thallomys moggi* Roberts, at Onderstepoort in March, 1927. This is a very variable species. The above specimens mainly differ from Ferris' description in the absence of hairs between the sclerites and pleurites on all the tergites and sternites and in size, the females measuring 1.47 mm. and the males 1.08 mm. in length, whereas Ferris states that the female measures 1.1 mm. and the male 0.95 mm.

2. HOPLOPLEURA ENORMIS ENORMIS Kellogg and Ferris.

H. enormis Kell. and Ferris, *Ann. Durban Mus.*, I, p. 155, pl. 16, f. 4-4e (1915).
H. enormis enormis (K. and F.) Ferris, *Stanf. Univ. Publ.*, II, ii, pp. 94-95, f. 57 (1921).

Several females and one male collected by the writer off *Lemniscomys spinalis* Thos. (bushveld striped mouse) at Sycamore on the 3rd November, 1927. Originally described from specimens taken off *Arricanthis dorsalis* = *L. spinalis* in Zululand.

3. HOPLOPLEURA BISERIATA Ferris.

H. biseriata Ferris, *Stanf. Univ. Publ.*, II, ii, pp. 103, 104, f. 64a (1921).

Two females taken off *Tateroma angolae*, a gerbille, in South-West Africa. This species was described from a single female taken off *Malacothrix typicus typicus* A. Smith (typical large-eared mouse) at Bothaville, Orange Free State.

Genus BATHYERGICOLA nov. gen.

Eyes absent. Antennae five-jointed, not sexually dimorphic. Thorax slightly wider than head, rounded posteriorly, without a sternal plate. Fore and mid legs equal in size, hind legs slightly larger; tibiae produced into a thumb-like process opposite the claw. Abdomen oval, each segment except the last with a row of long hairs on the dorsum and venter; tergal and sternal plates absent; pleural plates present on the second to sixth segments.

Hosts.—Rodents known as "mole rats" belonging to the family Bathyergidae.

Geno-type: *Bathyergicola hilli* nov. sp.

1. Bathyergicola hilli nov. sp.

(Figs. 6, 7, 7a, and 8.)

Female.—*Head* (Fig. 6) elongated, with the antennae situated a short distance behind anterior margin; forehead rounded in front with prominent prae-antennal angles; on the anterior margin there are four minute hairs, and two similar hairs behind them on the dorsum; post-antennal area with lateral margins sub-parallel; on each margin there are three short hairs, one above the other, and a long bristle at the latero-posterior angle; on the ventral surface there is a hair on each side just below the base of the antenna; occipital area entering a V-shaped groove in the thorax. Antennae with the first joint the largest; second joint slightly larger than each of the three apical joints, which are sub-equal in size.

Thorax with a short hair on each side in front, and one long bristle on each side of the meson behind. Ungues of fore and mid legs equal, much smaller than those of hind legs.

Abdomen ovate, the first to seventh segments each with a row of long hairs on the dorsum and venter; eighth tergite and sternite with two long hairs on the posterior margin; ninth tergite bare except for four minute hairs behind the posterior margin; ninth sternite with a patch of about nine long bristles and one spine on each below the gonopod, which is elongated and indistinct. Pleural plates similar, weakly developed, each with a long bristle and a short spine as shown in Fig. 7a. Seventh and eighth segments with two long bristles at each latero-posterior angle.

Male.—Differs from the female as follows: The eighth tergite has a row of four hairs; ninth tergite with about half a dozen small spines in the middle in front and a few minute hairs on each side beneath them; on the posterior margin there are six hairs and several minute ones close together. Male genitalia and genital plate as shown in Figure 8.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.3	0.16	0.27	0.18
Thorax	0.13	0.25	0.13	0.25
Abdomen	1.03	0.83	1.01	0.68
Total ...	1.46 mm.		1.41 mm.	

Holotype: a female.

Described from two females and two males taken off *Georychus hottentotus* at Pietermaritzburg, Natal, on the 22nd June, 1922, by Mr. Lawrence Hill, after whom we have much pleasure in describing this species.

2. Bathyergicola lawrensis nov. sp.

(Figs. 7b, 9, 10.)

Female.—Head (Fig. 9) elongated, forehead rounded in front, with a narrow transverse brown band on the dorsum in front and a similar band on each side above the base of the antenna; on the venter there is a short longitudinal band on each side of the mouthparts. On the dorsum of the prae-antennal area there is a transverse row of four short hairs in front of the transverse band and a minute one on each side above the lateral band; on the venter there are several minute hairs in the middle and four short hairs on each side, the two middle ones placed close together. Lateral margins of postantennal area very slightly rounded with five hairs, one above the other, the last being the longest. On the dorsum there is a minute hair on each side between the antennae, two short ones close together some distance below each, and another minute hair midway between these and the posterior margin; on the venter there is a short hair on each side between the antennae. Occipital area rounded. Antennae with a brown transverse band on each segment, the first being the largest, and the second is slightly larger than each of the remaining joints.

Thorax wider than head, with a long hair on each side of the meson in front of the posterior margin, which projects backwards in the middle on to the abdomen.

Abdomen ovate, first tergite with three irregular rows of long hairs in the middle and about ten long and short ones on each side; second to eighth tergites each with a single row of long hairs in the middle and one or two on each side; apical tergite with a narrow semi-circular brown band; first to seventh sternites each with a single median row of long hairs, and three or four similar hairs on each side; eighth sternite with a small patch of hairs in the middle; apical sternite with five hairs on each between the gonopods, and a tuft of hairs beneath each gonopod, which is long and narrow. Pleural plates on second segment each consisting of a small tooth-like lobe; the plates on the third to sixth segments resemble each other in shape and size (see Fig. 7b). Stigmata present on the third to eighth segment.

Measurements.

	Length.	Width.
Head	0.45	0.28
Thorax	0.16	0.46
Abdomen	1.5	1.36
Total	2.11 mm.	

Described from four females taken off *Bathyergus maritimus* Gmel., a mole rat occurring in the Cape Province. It is quite possible this species may ultimately have to be transferred to a new genus.

Genus LEPIDOPHTHIRUS Enderlein.

Lepidophthirus Enderlein, *Zool. Anz.*, XXVIII, pp. 43, 137 (1904).

1. LEPIDOPHTHIRUS MACRORHINI Enderlein.

L. macrorhini Enderlein, *Zool. Anz.*, XXVIII, pp. 46, 47, f. 1-5 (1904).

A number of males, females, and immature specimens taken off a giant seal (*Macrorhinus leoninus*) captured off the coast near Capetown (coll. R. F. Lawrence). This species was described from specimens taken off the same host on Kerguelen Island.

Sub-order MALLOPHAGA.

Family Menoponidae Mjöberg.

Genus Menopon Nitzsch.

Menopon Nitzsch, *Germar's Mag.*, iii, p. 299 (1818).

1. Menopon tumidum Piaget.

Menopon tumidum Piaget, *Pédicul., Suppl.*, p. 151, pl. 16, f. 5 (1885).

Menopon africanum Kellogg and Paine, *Bull. Ent. Res.*, p. 149, pl. 5, f. 3 (1911).

Menopon africanum transvaalensis Bedford, *Seventh and Eighth Reps. Dir. Vet. Res.*, Un. S. Afri., p. 716 (1920).

Several males and females taken off *Alopochen aegypticus* L. (Egyptian goose) at Tamanzu, South-West Africa, in March, 1923, and one female and one male from *Poecilonetta erythrorhyncha* Gm. (red-billed duck) in South-West Africa.

After having examined specimens from various geese and ducks, including *Plectropterus gambensis*, we have come to the conclusion that *M. africanum* Kellogg and Paine, which was desciibed from specimens taken off *P. gambensis* in the Sudan, is a synonym of *M. tumidum* Piaget, described from males and females taken off the same host. Piaget's description and figure differ mainly from Kellogg and Paine's in the presence in the female of two rows of hairs on the dorsum of each abdominal segment instead of only one row. We know that Piaget was not always reliable in describing and figuring the chaetotaxy of these insects, and as the males possess two rows of hairs on the dorsum of each abdominal segment, except the first two, we consider it highly probable that he had a male in front of him when he drew the chaetotaxy of the abdomen of the female he figured.

2. Menopon powelli Bedford.

Menopon powelli Bedford, *Seventh and Eighth Repts. Dir. Vet. Res.*, Un. S. Afr., pp. 714, 715, pl. 2, Fig. 1 (1920).

Several males and females collected from *Chaetopus adspersus* Waterh. (red-billed noisy francolin) and *Pternistis afer* Mull. (Angora red-necked francolin) on the Kunene River, S.W.A., in March, 1923.

Genus Numidicola Ewing.

Numidicola Ewing, *Journ. Wash. Acad. Sc.*, XVII, iv, p. 90 (1927).

1. Numidicola antennata (Kellogg and Paine).

Menopon antennatum Kellogg and Paine, Bull. Ent. Res., II, p. 150, pl. 5, f. 1 (1911).

Two females and one male taken off *Numida papillosa* (guinea-fowl) on the Kunene River, S.W.A., in March, 1923. It has also been recorded (*Fifth and Sixth Repts. Dir. Vet. Res.*, Un. of S. Afri., p. 718, 1919) taken off *Numida coronata* in the Transvaal, but the skin of the bird from which the specimens were collected is in the Transvaal Museum and proves to be *N. papillosa transvaalensis* (Transvaal guinea-fowl).

Genus COLPOCEPHALUM Nitzsch.

Colpocephalum Nitzsch, *Germar's Mag.*, iii, p. 298 (1818).

1. COLPOCEPHALUM VITTATUM Rudow.

Colpocephalum vittatum Rudow, *Zeit. f. ges. Nat.*, XXVII, p. 469 (1866).

Three females and one male taken off *Ardeola ralloides* Scop. (squacco heron) on the Kunene River, S.W.A., in March, 1923. A second species, *C. zonatum*, has also been described by Rudow (*Zeit. f. ges. Nat.*, XXXIV, p. 391, 1869), taken off the same host.

Genus HELEONOMUS Ferris.

Heleonomus, Ferris, *Canad. Ent.*, p. 305, Sept., 1916.
This genus includes six species found on cranes.

1. HELEONOMUS MIANDRIUM (Kellogg).

Colpocephalum miandrium (part) Kellogg, *Rept. Kilimand. Exped.*, No. 15, iv, p. 53 (1908).

One male and one female taken off *Balearica regulorum* Benn. (crowned crane) on the Kunene River, S.W.A., in March, 1923. The female described by Kellogg has since been named *H. confusus* by Ferris (*Canad. Ent.*, p. 307, Sept., 1916). Kellogg's specimens were taken off *B. regulorum gibbericeps* in East Africa.

Genus BUCEROPHAGUS nov. gen.

Head wider than long; forehead gradually narrowing from the base to the apex, the ventral portion projecting slightly beyond the dorsum in front; antennal sinuses deep; mandibles situated well forward; on the ventral surface above the middle there is a small plate as shown in Figure 11. Mesonotum separated from metanotum. Abdomen elongate-oval in the female, oval in the male, with well-developed tergal and sternal plates and pleurites. Male genitalia apparently characteristic, as shown in Figure 12. Hind femora with a patch of about 45 hairs on the venter, and five strong short hairs on the posterior margin; similar hairs also present on posterior margins of mid-femora.

Genotype: *Bucerophagus africanus* nov. sp.

Colpocephalum productum Nitzsch, found on *Buceros abyssinicus*=*Bucorvus abyssinicus* Bodd., should also be included in this genus. Owing to the incomplete description given, it is impossible to state to what extent it differs from the new species described here, but judging from Giebel's figures of the male and female of *productum* the two appear to be distinct.

BUCEROPHAGUS AFRICANUS nov. sp.
(Figs. 11, 11a, 12.)

FEMALE.—Head and thorax light brown with darker markings of the same colour; legs pale with dark marginal bands; abdomen pale with dark brown bands and pleurites.

Head as shown in Figure 11.

Thorax.—The prothorax is winged with seven hairs of various length on each side, and eight long hairs on the posterior margin; transverse band on dorsum long and narrow with a long hair at each end. Mesonotum gradually broadening from base to apex, with a row of eight long hairs on the posterior margin. Metanotum same shape as mesonotum, and consisting of three plates, a broad median one and two narrow lateral plates, the former being separated from the latter by a narrow line; on the lateral margins of the median plate there are four hairs, and on the posterior margin of the metanotum there is a row of 14 long hairs and a short or minute hair between each long one. On the ventral surface there is a well marked median plate between the mid and hind coxae (see Figure 11a). Above this plate there is a short longitudinal plate on each side of the middle line, and above each of these a small patch of short hairs.

Legs.—The coxae are sparsely clothed with short hairs, and coxae i also have a longish hair at the inner posterior angle; fore and mid coxae widely separated.

Abdomen elongate-oval, being broadest at the fourth segment. The tergites each with a brown transverse band and a row of thirty longish hairs on the posterior margin, the outer hair on each side being considerably longer than the others; above each outer hair there are two or three short hairs and above these the spiracle; on the apical tergite the plate covers the entire segment, and there are several short and long hairs on the posterior and lateral margins. On each sternite there is a brown transverse band covered with short hairs, the hairs being more numerous on each side, especially on the fourth and fifth sternites. The sternal plates are shorter than the tergal plates, the latter separated from the pleurites, which are beset with short hairs and longer ones on the posterior margins, by a narrow pale line.

MALE.—The male resembles the female, except that the abdomen is not so elongated, and is widest at the fifth segment. The apical tergite has a transverse row of about fourteen hairs in the middle, and four longer ones on each side on the posterior margin. On the apical sternite there are ten long hairs forming two rows on each side, and about six short hairs between them in the middle. Genitalia as in Figure 12.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.45	0.58	0.43	0.56
Pronotum ...	0.26	0.5	0.26	0.5
Mesonotum ...	0.11	0.5	0.11	0.5
Metanotum ...	0.16	0.71	0.15	0.66
Abdomen	2.05	1.15	1.48	1.03
Total ...	3.03 mm.		2.43 mm.	

Holotype: a female.

Described from females and males taken off specimens of *Bucorvus schlegeli* Roberts (South African ground-hornbill) in the

Zoological Gardens, Pretoria, in March, 1922; at Mafa, South-West Africa, in February, 1923, and in the gardens at Groot Schuur, Capetown, in November, 1923. The bird in the zoological gardens at Pretoria came from Rhodesia.

Genus TRINOTON Nitzsch.

Trinoton Nitzsch, *Germar's Magazin*, iii, p. 300 (1818).

1. TRINOTON ACULEATUM Piaget.

T. aculeatum Piaget, *Pédiculines*, *Suppl.*, p. 136, pl. 15, f. 1 (1885).

Females and males taken off *Sarkidiornis melanotus africanus* Eyt. (knob-billed duck) at Oncka, S.W.A., in March, 1923, also from *Dendrocygna viduata* L. (white-faced duck) at Tamanzu, S.W.A., in March, 1923.

2. TRINOTON ANSERINUM (Fabr.).

Pediculus anserinus Fabr., *Syst. Antl.*, p. 345 (1805).

Females and males taken off *Alopochen aegyptiacus* L. (Egyptian goose) at Tamanzu, S.W.A., in March, 1923, and two immature specimens from *Plectropterus gambensis* Linné (spurwing goose) on the Kunene River in March, 1923.

3. TRINOTON QUERQUEDULAE (Linné).

Pediculus querquedulae Linné, *Syst. Nat.*, p. 621 (1758).

Males and females collected off *Sarkidiornis melanotus africanus* Eyt. (knob-billed duck) at Oncka, S.W.A., in March, 1923.

Genus PSEUDOMENOPON Mjöberg.

Pseudomenopon Mjöberg, *Arkiv. f. Zoologi*, VI, p. 50 (1910).

1. PSEUDOMENOPON ROSTRATULAE Bedford.

Pseudomenopon rostratula Bedford, *Fifth and Sixth Repts. Dir. Vet. Res.*, Un. S. Afr., pp. 722-723, pl. II, f. 9 (1919).

Two females taken off *Rostratula benghalensis* (painted snipe).

This species was described from specimens taken off the same host in the Rustenburg District, Transvaal.

Family LAEMOBOTHRIIDAE Mjöberg.

Genus LAEMOBOTHRION Nitzsch.

Laemobothrion Nitzsch, *Germar's Magazin*, III, p. 301 (1818).

1. LAEMOBOTHRION TITAN Piaget.

Laemobothrion titan Piaget, *Pédiculines*, p. 578, pl. 49, f. 1 (1880).

Laemobothrion africanum Kellogg, *Schwed. Zool. Exp. Kilimanjaro*, p. 47, pl. 7, f. 6 (1910).

Laemobothrion gypsis Kellogg, *Ent. News*, XVII, p. 63 (1906).

Four females, one male, and one immature specimen taken from *Buteo vulpinus* Gloger (*B. desertorum*) (steppe buzzard) at Kaoko, Otavi, S.W.A., in March, 1926. In the Veterinary Research Laboratory collection there are specimens taken off the following hosts: *Gyps coprotheres* Forst. (*G. kolbei*) (Cape vulture), Rustenburg District, Transvaal, 4th October, 1917; *Pseudogyps africanus fulleborni* Erl. (southern white-backed vulture), Rustenburg District, 10th August, 1917; *Pterolestes rufofuscus* Forst. (jackal buzzard); *Nisaetus spilogaster* Bp. (African hawk-eagle), Rustenburg District, 3rd October, 1917; *Milvus aegyptius parasitus* Daud. (Cape kite), Pietermaritzburg, Natal, 2nd January, 1910 (coll. L. Hill); and *Pandion haliaetus* L. (osprey). We have also seen specimens from *Pteroaetus verreauxi* Less. (black eagle), Mtabamhlopi, Natal, 17th October, 1921 (coll. L. Hill). We have no hesitation in sinking both *L. africanum* Kellogg and *L. gypsis* Kellogg as synonyms of *titan*. The former was described from specimens taken off *Pseudogyps africanus* in East Africa, and the latter from specimens taken off *Gyps coprotheres* in the Transvaal. The immature forms of *titan* have the temples rounded instead of angulated, and the specimens from which Kellogg drew up his description of *africanum* were certainly immature. Kellogg stated that the main difference between *gypsis* and *titan* was to be found in the abdominal markings, but these markings vary according to the age of the specimens. Too great a care cannot be exercised in describing highly chitinized species, such as species of *Laemobothrion*, *Trinoton*, *Dennyus*, and certain species of *Esthiopterum*, such as *E. diomedeae* Fabr., etc., as these species vary tremendously according to age; not only are the immature forms very distinct from the adults, but young adults may also appear quite distinct from fully matured adults. Not only do these species vary according to age, but the time they are left in a clearing media may also affect their general appearance.

2. Laemobothrion tinnunculi (Linné).

Pediculus tinnunculi Linné, *Syst. Nat.*, p. 612 (1758).

One male taken off *Cerchneis rupicola* Daud. (Cape kestrel) at Haenertsburg, Transvaal, 15th July, 1926 (Lawrence Hill).

Family Trichodectidae Burmeister.

In the majority of species of Trichodectidae the metanotum is divided, and in some species, notably *Trichodectes interruptofasciatus* Kell. and Ferris and *T. ovalis* Bedford, the posterior division appears to belong to the abdomen, and in describing the latter and other species of Trichodectidae (*Thirteenth and Fourteenth Repts. Dir. Vet. Educ. and Res., pp.* 841-857, 1928) we erroneously referred to this portion of the thorax as the first abdominal tergite.

Cummings (*Proc. Zool. Soc.*, London, p. 283, 1916) states that in *Eutrichophilus setosus* and *E. coëndu* the male genitalia are quite of the same type, and that another type is formed by *Trichodectes latus*, *T. crassus*, and *T. pinguis*, and still another type may be seen in the male genitalia of *Trichodectes gastrodes* Cummings, *T. mephitidis* Osborn, *T. geomydis* Osborn, and *T. interruptofasciatus* Kell. and Ferris. The male copulatory apparatus will probably ultimately be found to be of generic importance in this family, although it must be admitted that at least in a few closely allied species it differs markedly. Another important generic character is the shape of the head and chitinous bands on the forehead.

Genus Trichodectes Nitzsch.

Trichodectes Nitzsch, *Germar's Magazin*, III, p. 294 (1818).

1. Trichodectes cooleyi nov. sp.

(Fig. 13.)

Female.—*Head* wider than long, the anterior margin rounded, without a median notch. Antennal bands dark, enlarged in front of the trabecula-like processes, which are prominent, and also in front where they are separated by a narrow clear space. Antennal sinuses shallow. Ocular projections prominent. Temples rounded with a narrow marginal band; the bands being joined together by a broader basal band. On the anterior margin there are four hairs on each side, the two front ones being the longest. On the dorsum there are eight hairs as in the male (Fig. 13). On the venter there is a broadish dark band extending backwards and outwards on each side of the forehead; between each band and the margin there is a row of three short hairs. On the gular region there is a chitinized plate which is almost as long as it is broad. Occipital bands indistinct. Antennae with the basal joint the shortest; second and third segments subequal.

Prothorax very short and broad, with a transverse brown band which is constricted in the middle; on the posterior margin there is a minute hair on each side of the meson.

Metathorax broader than the prothorax, with a short hair on each side and two minute ones above it.

Abdomen pale, oval, widest at the third and fourth segments; sutures for the most part indistinct; spiracles extremely small, present on the second to fourth segments. On the lateral margins of the second to eighth tergites and sternites there are one or two inconspicuous short hairs. On the apical tergite there is a very narrow brown transverse plate, and below it a transverse row of six short hairs, and two more below them on the posterior margin. On the apical sternite there is a short hair on each side of the meson below the middle of the segment. The gonopods are very narrow, slightly curved, and do not project beyond the margin.

Male (Figure 13).—The male resembles the female, except that the antennae are slightly longer, and the abdomen is of a different shape and has a narrow chitinized transverse band on the fourth, fifth, and sixth sternites; also a short, narrow, transverse band at the latero-anterior angles of the abdomen.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.28	0.36	0.26	0.31
Prothorax ...	0.06	0.26	0.05	0.2
Metathorax ...	0.08	0.31	0.06	0.25
Abdomen ...	0.66	0.61	0.54	0.48
Total ...	1.08 mm.		0.91 mm.	

Holotype: a male.

Described from males and females taken off a suricate (*Suricata suricatta hamiltoni*) shot in the Pretoria District by Prof. R. A. Cooley on the 18th September, 1928.

This species is quite distinct from other species known to occur on animals belonging to the family Viverridae. It will probably ultimately have to be placed in another genus, but for the time being we are obliged to place it in *Trichodectes* as we have not had an opportunity of comparing it with *T. canis* Degeer, the type of the genus.

2. TRICHODECTES OVALIS Bedford.

T. ovalis Bedford, *Thirteenth and Fourteenth Repts. Dir. Vet. Res.*, S. Afr., II, pp. 481, 482, pl. II, f. 4, pl. III, f. 7 (1928).

Three females taken off *Ictonyx striatus* (stink muishond) in S.W.A. We have also seen specimens collected on the same host in Natal by Mr. Lawrence Hill. This species was described from specimens taken off *Poecilogale albinucha* at Onderstepoort, near Pretoria.

Genus TRICHOLIPEURUS nov. gen.

Head.—Long and narrow; forehead with lateral margins straight or almost so, wider behind than in front. Frontal sinus narrow, emarginated, with two plates separated by a narrow clear space; antennal bands extending forward to the latero-anterior angles; on the ventral surface of forehead a pair of narrow admedian bands extending backwards in a straight line either from the latero-anterior angles or base of marginal plate to the mandibles, where they turn outwards. Temples with lateral margins either almost straight or slightly convex, slightly wider in the female than in the male. Occipital bands elongated, almost straight and parallel; similar bands present on venter. Occiput with a marginal band connecting the occipital bands together. Antennal sinuses shallow in the female, wide in the male. Antennae 3-jointed in both sexes; first joint of male elongated, as long as or longer than the second and third together; third joint of male with two small spines on the inner surface near the apex.

Thorax not winged, widening very gradually from base to apex. Pronotum short with a pair of stigmata at the latero-posterior angles. Between the fore and mid coxae a transverse acetabular bar running in from the side.

Abdomen elongated and narrow, usually with crenulated margins. Segments with a transverse brown band and a row of minute pustulated hairs on the dorsum and venter. (The males of *T. trabeculae* and *T. reduncae* possess two transverse bands on each of the tergites ii to vi.) Stigmata large, present on segments ii to vii.

Species found on antelopes.

Genotype: *Tricholipeurus aepycerus* nov sp.

This genus also includes *T. cornutus* Gerv., *T. lineatus* Bedford, *T. bedfordi* Hill, and *T. reduncae* nov. sp.

1. Tricholipeurus aepycerus nov. sp.
(Figs. 14, 15, 18.)

Male.—*Head* (Fig. 15) light brown, paler in front and behind the mandibles, with reddish-brown bands and mandibles. Forehead with about nine hairs on each lateral margin, and a transverse row of ten minute pustulated hairs on the dorsum, two on each side below the lateral ones, and two more on each side near the base of the trabecula-like process. Between the antennae there are two more on each side. On each temple there are seven to eight minute pustulated hairs, and in the middle of the hind head there is a transverse row of six. On the ventral surface there are three on each side of the forehead, and four more on each side above the base of the mandibles. Trabecula-like processes triangular. Temples with a narrow marginal band, the band being wider and darker in front of the eye. Signature on gular region triangular. First segment of antennae broad, longer than the second and third together, which are of approximately equal length; third joint with a row of very minute spines along the inner edge.

Thorax same colour as head, with a narrow lateral dark band.

Pronotum with two minute pustulated hairs on each side of the meson, four on each side on the posterior margin, and one on each lateral margin. *Metanotum* with a row of 23 similar hairs on the posterior margin. The acetabular bars extend back and widen out between the mid coxae. Above the hind coxae there is a small pustulated hair on each side of the middle.

Legs with the mid tibiae longer and narrower than the fore and hind tibiae.

Abdomen elongated and narrow with crenulated lateral margins; white in colour with a brown transverse band and a row of minute pustulated hairs on the dorsal and ventral surfaces of each segment; tergite i with a narrow marginal band; tergites ii to vii with a short comma-shaped chitinous bar in front of each spiracle; close to these bars and nearer the middle there is a short, forked, chitinous spot. Spiracles large. Tergite viii with six hairs above the row on the posterior margin. Apical tergite with a narrow marginal band fringed with minute hairs on the posterior margin, and with six hairs in the middle. On the last three sternites there is a narrow longitudinal band on each side of the genitalia. Male genitalia as in Figure 14.

Female.—*Head* as in the male, except that the hind head is slightly wider. First joint of antennae slightly broader than the last two, as long as the second; third joint the longest.

Thorax and legs as in the male.

Abdomen without forked, chitinous spots on the tergites; tergite viii with two short pustulated hairs in the middle, and two more close together on each side between them and the lateral margin; apical segment bilobed with four hairs on each lobe; venter as shown in Figure 18.

Measurements.

	Male.		Female.	
	Length.	Width.	Length.	Width.
Head	0.6	0.42	0.61	0.48 at temples.
Prothorax ...	0.07	0.32	0.07	0.34
Metathorax ...	0.14	0.35	0.14	0.4
Abdomen ...	1.49	0.56	1.58	0.65
Total ...	2.3 mm.		2.4 mm.	

Holotype: the male.

Described from one male and one female taken off *Aepyceros melampus* (impala), Kunene River, S.W.A., March, 1923. It can be distinguished from *T. lineatus* Bedford and *T. bedfordi* Hill by the male genitalia, the large stigmata on the abdomen and comma-shaped markings above them, and also by the tibiae. In *lineatus* the mid and hind tibiae are narrow and equal in length, the mid being very slightly narrower than the hind tibiae. In *bedfordi* the mid and hind tibiae are equal in length and width, and shorter and broader than in *lineatus*.

Tricholipeurus trabeculae nov. sp.

(Figs. 17a, 17c, 19.)

Male.—*Head* light brown, paler in front of the mandibles, with reddish-brown bands and mandibles. Frontal plates with rounded outer margins. Forehead with three or four minute hairs on each lateral margin, and on the dorsum there are about 10 minute pustulated hairs between each antennal band and frontal plate, a transverse row of six below the plates, and six more on each side above the base of the antennae. Between the antennae there are two more on each side. On each temple there are about fourteen minute pustulated hairs, and in the middle of the hind head there is a transverse row of nine similar hairs. On the ventral surface there are several very minute pustulated hairs in the area between each antennal and admedian band, two larger ones on the latter band where it turns outwards, and one at the base of each antenna. Trabecula-like processes with an acute angle at their apices. First segment of antennae about twice as long as broad, about same length as the second and third together; apical joint very slightly longer than second, with a row of very minute spines along inner margin. Temples with a narrow marginal band, the band being wider and darker in front of the eye. Signature on gular region triangular.

Thorax same colour as head with a narrow lateral band.

Pronotum with two minute pustulated hairs on each side of the meson, four on each side on the posterior margin, and one on each lateral margin.

Metathorax with a narrow transverse band, which is interrupted in the middle where it turns forward, and a row of sixteen hairs on the posterior margin. The acetabular bars extend backwards and widen out between the mid coxae. Above the hind coxae there is a small pustulated hair on each side of the meson.

Legs with the mid and hind tibiae short and broad, the latter very slightly broader than the former.

Abdomen elongated with crenulated lateral margins; white in colour with dark brown bands. Tergite i with one transverse band and a row of short pustulated hairs. Tergite ii to vi each with two transverse bands and a row of hairs between them, the anterior bands being more highly chitinized at their antero-lateral margins, and the posterior bands are smaller. Tergites vii and viii each with one transverse band; tergite ix with a very narrow band on the posterior margin and a few short hairs. Pleural plates indistinct, with a few pustulated hairs. Stigmata of medium size. Sternites each with one transverse band and one row of hairs, except on the first. Sternites vi and vii also with a narrow longitudinal band on each side of the middle.

FEMALE.—*Head* wider at the temples than in the male. Trabecula-like processes normal. First joint of antennae the shortest, slightly broader than the last two, which are sub-equal in length. Thorax and legs as in the male. Abdomen with a transverse band and one row of hairs on each dorsal and ventral segment, except the last. Apical sternite with two plates as shown in Fig. 19. The pleurites more distinct than in the male.

Measurements.

	Male.		Female.	
	Length.	Width.	Length.	Width.
Head	0.41	0.32	0.42	0.36 at temples.
Prothorax ...	0.08	0.25	0.10	0.28
Metathorax ...	0.10	0.27	0.11	0.31
Abdomen ...	0.93	0.38	1.00	0.45
Total ...	1.52 mm.		1.63 mm.	

Holotype: a male.

Described from two males and two females taken off *Redunca fulvorufula* (mountain reedbuck), Mfongosi, Zululand. This species has erroneously been recorded under the name *T. cornutus* Gerv., but it is very distinct; the latter being more closely allied to *T. aepycerus*. From *T. lineatus* Bedf., *T. aepycerus*, and *T. bedfordi* the females of *trabeculae* can be distinguished by the apical sternite, and the males by the shape of the trabeculae, presence of two transverse bands on the median tergites, and also by the male genitalia.

Tricholipeurus reduncae nov. sp.

(Figs. 16, 17, 17b.)

This species is very closely allied to *trabeculae*, from which it can be distinguished as follows:—

(1) Head of female broader, being about as broad as long.

(2) Bands darker, especially the transverse bands on the abdomen.

(3) Pleurites well developed and stigmata, especially in the male, larger (compare Figs. 17 b and c, both drawn to same scale).

(4) Male genitalia longer and endomeres slightly different in shape (Figure 17).

In the male the posterior bands on tergites ii to vi are darker than the anterior bands, the fifth and sixth being interrupted in the middle.

Measurements.

	Male.		Female.	
	Length.	Width.	Length.	Width.
Head	0.40	0.35	0.41	0.40
Prothorax ...	0.10	0.3	0.1	0.30
Metathorax ...	0.11	0.31	0.11	0.38
Abdomen ...	1.00	0.45	1.11	0.63
Total ...	1.61 mm.		1.73 mm.	

Holotype: a male.

Three males and several females taken off *Redunca arundinum* Bod. (reedbuck) by the author at Emakosini, N. Zululand, on the 29th October, 1924. These specimens have also been erroneously recorded under the name *T. cornutus* Gerv.

Genus Bovidoecus nov. gen.

Head either very slightly broader than long, or almost as broad as long. Forehead rounded, either very slightly convex or concave in front, with a narrow marginal band which is slightly wider in front and interrupted in the middle by a very narrow pale area, except in *limbatus*. Temples rounded. On the venter of the forehead there is a pair of admedian bands, each extending backwards and outwards from the anterior margin to the lateral margins. Occipital bands project forwards and then outwards to the latero-posterior margins of forehead; similar bands present on the venter. (In *limbatus* these bands are absent.) Occiput with a marginal band. Antennal sinuses shallow in the female, wider in the male. Antennae situated either in the middle of the head or well forward (*limbatus*); 3-jointed, basal joint similar in both sexes, except in *oris* it is slightly larger in the male, much shorter than second and third joints together; third joint of male with a pair of very small spines on the inner surface near the apex.

Thorax narrower than head. Prothorax with rounded lateral margins. Metathorax wider in front than behind; lateral margins rounded anteriorly. Between the fore and mid coxae a transverse acetabular bar running in from the side.

Abdomen ovate. Segments with a transverse brown band and a row of small pustulated hairs on the dorsum and venter; also a few similar hairs sometimes present above the row and at the sides between the pleurites and bands. (In *limbatus* the hairs are very numerous.) Stigmata present on segments ii to vii. Pleurites well developed. Species found mainly on Bovidae, also on equines.

Genotype: *T. bovis* Linné.

In this genus must also be included *T. caprae* Gurlt., *T. painei* Kellogg and Nakayama, *T. ovis* Linné, *T. equi* Linné, *T. peregrinus* Tasch., and *T. limbatus*. The relationship of the last named to the other species appears to be the same as is the relationship between *Syrrhaptoecus brevifrons* Waterston and the other known species of *Syrrhaptoecus*. *T. harrisoni* Cummings found on *Connochaetes gnu* (black wildebeest) may be distinct, but should be included here for the present. The apex of the abdomen of the male is very different, and the male genitalia appear to be of a distinct type.

Genus FELICINIA nov. gen.

Small species (males 0.92—1.35 mm.; females 1.15—1.56 mm.) *Head* about as long as broad; forehead conical, slightly emarginated in front, antennal bands broad, separated in front by a clear space, and projecting backwards for a short distance on each side of the median area. Trabecula-like processes prominent. Antennal sinuses shallow in the female, usually broader in the male. Temples rounded with a narrow marginal band. Occipital bands dark at their bases, and becoming indistinct in the middle of the head; similar bands present on the venter extending forwards to the latero-posterior margins of the forehead. First joint of antennae either equal in both sexes (*subrostratus* and *acutirostris*) or larger in the male than in the female; third joint of male either with or without a pair of minute spines on the inner margin near the apex. Signature on gular region triangular.

Prothorax narrower than the head with rounded lateral margins and a pair of stigmata at the latero-posterior angles.

Metathorax about as wide as the head and shorter than the prothorax with rounded lateral margins. Between the fore and mid coxae a transverse acetabular bar running in from the side and joined together in the middle; each bar also has a branch extending backwards a short distance between the mid coxae.

Abdomen differs in shape in both sexes; in the female it is oval, and the last segment is bilobed at the apex; in the male it is either rounded or pointed posteriorly. Tergites i to vii each with a transverse brown band, these bands being duplicated on the median segments in some males. The median sternites each with a row of minute pustulated hairs and with or without a band. Pleurites only developed on the first to third segments. Stigmata present on the second to fourth segments.

Species found on Felidae and Viverridae.

Genotype: *T. subrostratus* Nitzsch.

In this genus must also be included: *T. acutirostris* Stobbe, *T. cynictis* Bedf., *T. calogaleus* Bedf., *T. rammei* Stobbe, *T. mungos* Stobbe, *T. inaequalis* Piaget, and *T. viverriculae* Stobbe.

Family PHILOPTERIDAE Burmeister.

Genus GONIODES Nitzsch.

Goniodes Nitzsch, *Germar's Magazin*, III, p. 293 (1818).

1. GONIODES NUMIDAE Mjöberg.

G. numidae Mjöberg, *Arkiv. f. Zoologi*, vi, p. 103, f. 60, 61 (1910).

Two females taken off *Numida papillosa* (guinea-fowl), Kunene River, S.W.A., March, 1923. We have also recorded it taken off *Numida coronata* at Bridgewater, Transvaal, but the skin of the bird from which the specimens were collected is in the Transvaal Museum, and proves to be *Numida papillosa transvaalensis* Neum.

2. GONIODES SCLEROPTILUS nov. sp.

(Fig. 20.)

Two females and one male taken off *Scleroptila gariepensis pallidior* Neum. (Ovambo partridge) on the Kunene River, S.W.A., in March, 1923.

This new species resembles *G. dispar* Nitzsch, *G. flaviceps* Rudow, and *G. assimilis* Piaget in the shape of the head, thorax, and abdomen. *G. dispar*, found on *Perdix perdix* (European partridge), can be distinguished by the lateral bands on the abdomen not having an appendage in front. In *G. assimilis*, which was described from specimens taken off *Chaetopus capensis* (Cape noisy francolin), only the lateral bands on the last segment have an appendage, and in *G. flaviceps*, found on *Perdix rubra* (European red-legged partridge), and *G. scleroptilus* the bands on all the segments have an appendage. In *G. flaviceps* these bands terminate in a circular spot, whereas in *G. scleroptilus* the spots are rectangular and lie beneath the bands. Male genitalia as in Figure 20.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.85	1.1	0.71	0.93
Prothorax ...	0.13	0.5	0.11	0.42
Metathorax ...	0.33	0.71	0.3	0.6
Abdomen ...	1.5	1.48	1.03	1.18
Total ...	2.81 mm.		2.15 mm.	

3. GONIODES PTERNISTIS nov. sp.

(Fig. 21.)

Several males and females taken off *Pternistis swainsoni* A Smith (Swainson's red-necked francolin) in the Zoological Gardens Pretoria, on the 4th November, 1925. This species is very closely allied to *G. scleroptilus*, the males being distinguished by the genitalia, and the females by the absence of a transverse row of small spines on the ventral surface of the seventh abdominal segment. The female of *G. flaviceps* also possesses a row of similar spines on the seventh sternite, but in this species they are arranged in a semicircular line and number about eight, whereas in *G. scleroptilus* there are about fourteen spines arranged in an almost straight line in front of the posterior margin.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.86	1.15	0.71	0.93
Prothorax ...	0.13	0.48	0.11	0.4
Metathorax ...	0.33	0.7	0.28	0.56
Abdomen ...	1.5	1.45	1.00	1.18
Total ...	2.82 mm.		2.10 mm.	

Genus GONIOCOTES Burmeister.

Goniocotes Burmeister, *Handb. der Ento.*, II, p. 43 (1838).

1. GONIOCOTES GALLINAE Retz.

One female taken off *Numida papillosa* (guinea-fowl), Kunene River, S.W.A., March, 1923. It has also been recorded by us taken off *Numida coronata* in the Transvaal, but the bird proved to be *N. papillosa transvaalensis* Neum.

2. GONIOCOTES GIGAS Taschenberg.

G. gigas Taschenberg, *Zeit. f. ges. Nat.*, LII, p. 104, Pl. 1, f. 10 (1897).

Males and females taken off *Numida papillosa* (guinea-fowl), Kunene River, S.W.A., March, 1923. We have also recorded it found on *N. coronata* at Bridgewater, Transvaal, the host proving to be *N. papillosa transvaalensis* Neum. It is a common parasite on the domestic fowl.

Genus LIPEURUS Nitzsch.

Lipeurus Nitzsch, *Germar's Magazin*, III, p. 292 (1818).

1. LIPEURUS LAWRENSIS nov. sp.

(Figs. 22, 23, 23a, 24.)

MALE.—*Head* (Figure 22) elongated, rounded in front; pale brown in colour with dark brown markings and black bands; on the venter of the forehead there are four hairs situated a short distance behind the anterior margin, and one on each side below them. Signature on gular region long and narrow and dark brown in colour.

Thorax pale with dark brown lateral margins. *Pronotum* narrower than the head with sides almost parallel, and a longish hair at each latero-posterior angle. Metanotum broader than the pronotum, the anterior third being narrower than the posterior two-thirds, with a narrow, dark, median, longitudinal band extending backwards from the middle on to the first tergite; at each latero-posterior angle there is a hair, and a short distance from each and slightly nearer the middle there are four long hairs in a small pale area. Sternal plate as in Figure 22a.

Abdomen with markings and hairs on the dorsum as shown in Figure 23. Sternites i-iii each with a brown sternal plate, and a transverse row of six hairs near the middle. On sternites iv to viii the plates are fused together, the plates on the fourth and fifth sternites each have six hairs, on the seventh there are two long pustulated hairs, and on the eighth there are three long hairs on each side of the meson. Genitalia with elongated basal plate extending forward to the third segment. Figure 23 shows the apex of the abdomen before mounting, and Figure 23a after mounting.

FEMALE.—Shorter but slightly wider than the male. Head and thorax as in the male. Antennae with the first joint short and narrow, the second joint the longest, the third slightly longer than either of the two apical joints.

Abdomen with markings and hairs on the dorsum as shown in Figure 24. Sternal plates smaller than those of the male and not fused together, except those on sternites vi and vii; each plate with a median row of four hairs. On sternites v to viii there is a narrow longitudinal band on each side between the plates and lateral margins; the bands being broadest on the seventh sternite where they are forked in front.

Measurements.

	Male.		Female.	
	Length.	Width.	Length.	Width.
Head	0.78	0.55	0.83	0.58
Prothorax ...	0.25	0.45	0.23	0.46
Metathorax ...	0.46	0.7	0.45	0.72
Abdomen ...	2.45	0.8	2.11	0.95
Total ...	3.94 mm.		3.62 mm.	

Holotype: the male.

Described from one male, one female, and one immature specimen taken off a guinea-fowl, *Numida papillosa*, on the Kunene River, S.W.A., in March, 1923. Three other species have been described from guinea-fowl, namely, *L. numidae* Denny, *L. differens* Piaget, and *L. numidianus* Mjöberg, but this new species can easily be distinguished from these, *inter alia*, by the abdominal markings.

2. LIPEURUS PTERNISTIS nov. sp.

(Figs. 25, 25a, 26.)

FEMALE.—*Head* (Fig. 25) elongated, rounded in front, pale brown in colour with brown markings and dark brown marginal bands; on the venter of forehead there are four hairs situated a short distance behind the anterior margin, and two more, one on each side, below them. Signature on gular region about twice as long as broad.

Thorax pale with dark brown lateral margins. *Pronotum* narrower than the head with sides almost parallel, and a long hair at each latero-posterior angle. *Metathorax* broader than prothorax, the anterior third being narrower than the posterior two-thirds; at each latero-posterior angle there is a hair, and on the posterior margin there are two pairs on each side, each pair situated in a small pale area. Sternal plate as in Figure 25a.

Abdomen with markings and hairs on the dorsum as shown in Figure 26. Sternites i to v each with a transverse row of six hairs in the middle. Sternite vi with a short, median, brown, transverse band and a pale brown longitudinal blotch on each side of it; on the posterior margin of the band there is a pair of short admedian hairs. Sternite vii with a longitudinal chitinous plate on each side between the meson and lateral margins, each plate terminating in a long dark brown point which projects on to the eighth sternite; between the plates there are two longish hairs in front and several minute ones behind; apical sternites with several minute hairs and a short thick one on each side below the plates on the seventh sternite.

MALE.—*Head* and *thorax* as in the female. Antennae with the first joint about twice as long as the second and nearly twice as broad; second joint longer than the third and nearly as long as the fourth and fifth together; third joint with an appendage.

Abdomen narrower than in the female. Tergites i to vi each with a complete transverse plate, which is narrowest in the middle, and a short, narrow plate beneath it; the anterior plates are brown in the middle and pale at the sides, and the posterior plates dark brown in colour, those on tergites i, v, and vi being interrupted in the middle. The anterior plate on the first tergite has two hairs in front and a row of six behind, and on the second to sixth segments each anterior plate has a row of four hairs on the posterior margin. Tergites vii and viii each with a complete transverse plate, that on the seventh with a row of four hairs, and on the eighth the plate is wide with two short hairs in front; beneath the plate there are one long and two short hairs on each side. Plate on apical tergite pale in the middle. Sternite i with a small pale brown band in the middle which has a row of four hairs. Sternites ii to vi each with a pale brown transverse band and a row of four to six hairs. On sternites vii and viii there is a median longitudinal band which joins the band on the sixth sternite; on the seventh sternite the band has a long pustulated hair on each side. Pleurites dark brown. Male genitalia indistinct; basal plate short.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.55	0.4	0.51	0.36
Prothorax ...	0.11	0.28	0.11	0.26
Metathorax ...	0.18	0.43	0.18	0.38
Abdomen ...	1.26	0.63	1.18	0.45
Total ...	2.10 mm.		1.98 mm.	

Holotype: a female off *P. swainsoni*.

Described from two females and two males taken off *Pternistis swainsoni* A. Smith (Swainson's red-necked francolin) in the Zoological Gardens, Pretoria, on the 4th November, 1925, also two females taken off *Pternistis afer* Mull. (Angola red-necked francolin), Kunene River, S.W.A., in March, 1923. This new species appears to be closely allied to *L. heterogrammicus* Nitzsch found on *Perdix perdix* (European partridge), from which it can be distinguished by the abdominal markings.

Genus PHILOPTERUS Nitzsch.

Philopterus Nitzsch, *Germar's Magazin*, III, p. 288 (1818).

Docophorus Nitzsch, *ibid.*, III, p. 289 (1818).

1. PHILOPTERUS ROSTRATUS (Nitzsch).

Docophorus rostratus Nitzsch in Burmeister, *Handb.*, II, p. 427 (1838).

Females and males from *Typo alba affinis* (Cape barn owl), Ondonga, S.W.A., March, 1923. It has also been recorded taken off the same host in the Transvaal, and from *Bubo africanus africanus.*

Genus ANATOECUS Cummings.

Anatoecus Cummings, *Proc. Zool. Soc.*, London, p. 653 (1916).

1. ANATOECUS ICTERODES (Nitzsch).

Docophorus icterodes Nitzsch in Burm., *Handb.*, ii, p. 424 (1838).

Anatoecus icterodes (Nitzsch) Cummings, *Proc. Zool. Soc.*, Lond., pp. 655-657, f. 7B, 10, 12 (1916).

Scopoli's name *dentatus* should be discarded and the above name used for the common species found on ducks.

One female and one male taken off *Dendrocygna bicolor* L. (whistling duck) at Tamanzu, S.W.A., March, 1923; also one female from *Paecilonitta erythrorhyncha* Gm. (red-billed teal).

Genus NEOPHILOPTERUS Cummings.

Neophilopterus Cummings, *Proc. Zool. Soc.*, London, p. 660 (1916).

1. NEOPHILOPTERUS ABDIMIUS nov. sp.

(Figs. 27, 28, 29.)

FEMALE (Fig. 27).—*Head* very slightly broader at the temples than long. Signature medianly excised in front and with a strong dark chitinous process at each latero-posterior angle. Just inside each angle there is a longish hair, and on the venter there is an admedian hair in the middle. Clypeus with two hairs at each latero-posterior angle, two on the venter on each side above the angle, and one more above these. Behind the clypeus there is a hair on each side on the venter and one on the dorsum near the base of the antennae. Eye conspicuous, with a hair. Antennae with the first joint about as broad as long, second joint the longest, third, fourth, and fifth subequal in length, the last two being slightly darker than the rest. Temples rounded with four long and two short hairs on the lateral margins, and a minute hair in the middle. In the area between the temples there are three minute hairs on each side on the dorsum. Occipital bands dark brown, slightly curved and joining the antennal bands near the trabecula-like processes. Occipital margin almost straight.

Thorax dark brown except for a clear median space. Pronotum with a longitudinal chitinous bar on each side of the middle and two hairs at each latero-posterior angle. Metanotum with very dark lateral margins; a spine and hair at each latero-posterior angle, and three long hairs close together on each side on the posterior margin. Between the fore and mid coxae there is a strong acetabular bar which continues forwards and backwards a short distance near the middle. Between the mid and hind coxae there is a narrow bar which continues backwards to the base of the metasternum. Mid coxae with a hair on the posterior margin. Between the mid coxae there is a long admedian hair, and two more beneath it.

Abdomen oval, each segment with two tergal plates on each side, the inner pairs separated in the middle by a clear space except on the eighth segment, and with two median transverse rows of hairs, except on the eighth segment, and two or three hairs at each latero-posterior margin. On the venter there is a single row of median hairs on the first six segments. Apical segments as shown in Fig. 29.

MALE.—Head and thorax as in the female. The inner tergal plates are not so large as in the female, and the last tergite has a narrow longitudinal plate on each side of the middle line, and a lateral plate which extends backwards in a narrow line. Male genitalia and genital plate as shown in Fig. 28.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	0.78	0.85	0.75	0.76
Prothorax ...	0.21	0.55	0.2	0.51
Metathorax ...	0.23	0.7	0.21	0.63
Abdomen ...	1.53	1.15	1.08	0.96
Total ...	2.75 mm.		2.24 mm.	

Holotype: a female.

Described from three females and two males taken off *Sphenorhynchus abdimi* Licht. (white-bellied stork) at Andoni, S.W.A., in March, 1923.

This new species appears to be closely allied to *N. unifasciatus* Piaget, *N. indicus* Piaget, and *N. episcopi* Kellogg. From the first two it can be distinguished by the tergal plates being markedly larger, and from *N. episcopi* by the genital plate of the female.

Genus IBIDOECUS Cummings.

Ibidoecus Cummings, *Proc. Zool. Soc.*, London, p. 663 (1916).

1. IBIDOECUS THRESKIORNIS nov. sp.

(Figs. 30, 31.)

FEMALE.—Head considerably broader than long. Forehead light brown, of moderate length; each signatural plate long, widest in the middle and terminating posteriorly in a strong dark chitinous process, in front of the middle on the ventral surface there is one longish hair. Clypeal bands well developed, each with three lateral hairs on the dorsum, two in front and one behind, and a longish hair close to the basal one but nearer the middle; on the venter there is a similar hair occupying the same position and also a lateral hair beyond the middle. On the dorsum there is a short hair above the base of the antenna, two more lateral hairs close together some distance above it, and one close to each chitinous process. Eye conspicuous, with a hair. Temples rounded, dark brown, with four long pustulated hairs on the lateral margins and a short pustulated hair in the middle. Area between the temples slightly paler in colour, with a row of four pustulated hairs, the inner pair being minute.

Prothorax much narrower than the head, almost parallel-sided and rectangular, with a dark brown rectangular plate on each side and four short hairs on the posterior margin. *Metathorax* with lateral margins rounded, a dark brown plate on each side, and a row of pustulated hairs on the posterior margin, which is very slightly rounded. Between the fore and mid coxae there is a strong acetabular bar which turns upwards for a short distance between the former. Between the mid and hind coxae there is a short bar on each side which broadens out at both ends. Fore coxae each with one hair, mid and hind coxae each with two hairs. Between the mid coxae there are two short hairs, and two longer ones between the hind coxae.

Abdomen oval; tergites one to six each with a lateral plate which is darker near the meson, and with two median transverse rows of hairs; on the first tergite there are two hairs above the rows. Seventh tergite with similar plates, but only one row of hairs. Eighth tergite with a complete transverse plate and a row of hairs set close together beneath it, the row being interrupted in the middle. On the ventral surface there is one median row of hairs and a round plate between the middle and lateral margin on the second to sixth segments. Apical sternites as shown in Fig. 31.

MALE.—Head and thorax as in the female. First tergite as in the female, except that there are two plates on each side, the inner pair being small; second to seventh tergites each with one irregular row of hairs. The tergal plates on the last three segments appear to be double, and on the last tergite occupy practically the whole of the segment; the middle pair having a fringe of hairs on their posterior margins. Genital plates and genitalia as shown in Fig. 30.

Measurements.

	Female.		Male.	
	Length.	Width.	Length.	Width.
Head	1.01	1.15	0.9	1.05
Prothorax ...	0.33	0.71	0.31	0.66
Metathorax ...	0.38	1.06	0.33	0.98
Abdomen ...	2.00	1.65	1.43	1.35
Total ...	3.72 mm.		2.97 mm.	

Holotype: a female.

Described from a number of females and males taken off *Threskiornis aethiopica* Lath. (sacred ibis) at Emakosini, Zululand, on the 29th October, 1924. This new species can be distinguished, *inter alia*, by the genital plates and male genitalia.

Genus DEGEERIELLA Neumann.

Degeeriella Neum., *Bull. Soc. Zool.*, France, XX, p. 59 (1906).
Nirmus Nitzsch, *Germar's Magazin*, III, p. 291 (1818), *nec* Hermann, 1804.

1. DEGEERIELLA COLYMBINA (Scopoli).

Pediculus colymbina Scop., *Ent. Carn.*, p. 384 (1763).

Two females taken off *Proctopus nigricollis gurneyi* Roberts (Cape eared grebe) at Tamanzu, S.W.A., in March, 1923; also several specimens occurring as stragglers en other water fowl.

2. DEGEERIELLA FUSCA (Nitzsch).

Nirmus fuscus Nitzsch in Denny, *Anoplur. Brit.*, p. 118, pl. 9, f. 8 (1842).

Females and males taken off *Pteroaetus verreauxi* Less. (black eagle) by Dr. R. F. Lawrence. No locality given.

Genus SYRRHAPTOECUS Waterston.

Syrrhaptoecus Waterston, *Proc. Zool. Soc.*, London, ii, pp. 337, 338 (1928).

This genus includes thirteen species found on sand-grouse (Pteroclidae).

1. SYRRHAPTOECUS DIGONUS Waterston.

S. digonus Waterston, *Proc. Zool. Soc.*, London, ii, p. 345, f. 3a, 8b, 10c (1928).

One male and one female taken off *Pterocles namaquus* Gm., Kunene River, S.W.A., March, 1923. This species was described from specimens taken off the same sand-grouse at Klipfontein, Little Namaqualand; Deelfontein, C.P.; and in the Rustenburg District, Transvaal. A second species, *S. brevifrons* Waterston (*Proc. Zool, Soc.*, London, ii, pp. 339-342, f. 1b. 7, 1928), has also been found on *P. namaquus.*

Genus Acidoproctus Piaget.

Acidoproctus Piaget, *Tijd. v. Ent.*, vi, p. 178 (1878).

This genus includes six species found on Anatidae.

1. Acidoproctus stenopygus (Nitzsch).

Lipeurus stenopygos Nitzsch in Giebel, *Ins. Epiz.*, p. 179, pl. 8, f. 6, 7 (1874).

Several males and females taken off *Alopochen aegyptiacus* L. (Egyptian goose) at Tamanzu, S.W.A., in March, 1923. It has also been recorded from *Plectropterus gambensis* (spur-winged goose) in the Transvaal and Sudan. Rudow has described *A. rostratus* from *Alopochen aegyptiacus*, which Harrison sunk as a synonym of *A. marginatus* Piaget. The latter was described from a female straggler collected on a gull, and is in our opinion undoubtedly an immature specimen of *A. bifasciatus*, which occurs on ducks and has been found on several species in S. Africa.

Genus Esthiopterum Harrison.

1. Esthiopterum asymmetricum (Rudow).

Lipeurus asymmetricus Rudow, *Zeit. f. ges. Nat.*, XXXV, p. 312 (1870).

Lipeurus gambensis Piaget, *Pédicul.*, *Suppl.*, p. 64, pl. 7, f. 1 (1885).

Several males and females taken off *Alopochen aegyptiacus* L. (Egyptian goose) at Tamanzu, S.W.A., March, 1923, and off *Plectropterus gambensis* L. (spur-winged goose) on the Kunene River, S.W.A., March, 1923. *E. asymmetricum* was described from specimens taken off the first-named host, and *E. gambense* from specimens off the second-named host; we consider them to be the same.

2. Esthiopterum obscurum (Rudow).

Lipeurus obscurus Rudow, *Zeit. f. ges. Nat.*, XXXVI, p. 125 (1870).

Males and females taken off *Thalassarche melanophrys* Boie (mollymawk) at Capetown, March, 1923.

3. Esthiopterum gurlti (Taschenberg).

Lipeurus gurlti Taschenberg, *Nova Acta*, XLIV, p. 151, pl. 5, f. 6 (1882).

Lipeurus lugubris Taschenberg, *ibid.*, p. 153, pl. 6, f. 9 (1882).

Males and females taken off *Daption capensis* (Cape sea-pigeon) at Capetown in November, 1923.

4. Esthiopterum gruis (Linné).

Pediculus gruis Linné, *Syst. Nat.*, p. 613 (1758).

Lipeurus ebraeus Denny, *Anoplur. Brit.*, p. 179, pl. 13, f. 5 (1842).

Lipeurus hebraeus Nitzsch in Giebel, *Ins. Epiz.*, p. 226, pl. 16, f. 5, 6 (1874).

L. hebraeus (Nitzsch) Piaget, *Pédiculines*, p. 326, pl. 27, f. 2 (1880).

L. hebraeus (Nitzsch) Taschenberg, *Nova Acta*, XLIV, p. 130, pl. 4, f. 4, 4a (1882).

A single female taken off *Balearica regulorum* Benn. (crowned crane), Kunene River, S.W.A., March, 1923. This species has been described from specimens taken off *Grus communis* = *G. grus* (European crane) and *G. pavonina* = *Balearica pavonina*.

Genus Harrisoniella nov. gen.

Species lipeuroid, large (females 9 mm., males 8.5 mm. in length). Head longer than wide, broader in the female than in the male. Clypeal signature broader than long, rounded in front and pointed posteriorly; pale brown with transverse striae along anterior margin, dark brown behind. Prothorax with sides parallel. Metathorax more than twice the length of prothorax. Sternal plate well developed, elongated. Abdomen of female with lateral plates on each tergite separated in the middle by a narrow space; tergites of male with a complete transverse plate; lateral margins dark in both sexes. Sternites of male and female each with a dark median transverse band. Male copulatory apparatus elongated, the basal plate long and narrow, about the same length as the remainder of the apparatus.

Genotype: *Esthiopterum diomedeae* (Fabricius).

1. Harrisoniella diomedeae (Fabricius).

Pediculus diomedeae Fabr., *Syst. Ent.*, p. 808 (1775).

Lipeurus ferox Giebel, *Zeit. f. ges. Nat.*, XXIX, p. 195 (1867).

L. ferox Giebel, *Ins. Epiz.*, p. 235 (1874).

L. ferox (Giebel) Piaget, *Pédiculines*, p. 333 (1880).

L. ferox (Giebel) Taschenberg, *Die Mallophagen*, p. 145, pl. 5, f. 1, 1a (1882).

L. ferox (Giebel) Kellogg, *New Mallophaga*, i, p. 127, pl. 9, f. 1, 2 (1896).

L. densus (Giebel) Kellogg, *ibid.*, p. 114, pl. 7, f. 1, 2 (1896).

One female and two immature specimens taken off *Thalassarche melanophrys* Boie (mollymawk) at Capetown, July, 1923.

Genus Docophoroides Giglioli.

Docophoroides Giglioli, *Quart. Journ. Micr. Sci.*, IV, p. 21 (1864).

Eurymetopus Taschenberg, *Nova Acta*, Halle, XLIV, p. 182 (1882).

This genus includes five species occurring on Diomedeidae.

1. Docophoroides harrisoni Waterston.

D. harrisoni Waterston, *Ent. Month. Mag.* (3), iii, p. 99 (1917).

A number of females, males, and immature forms taken off *Thalassarche melanophrys* Boie (mollymawk) at Capetown in July, 1923. This species was described from specimens taken off the same host in South Africa.

2. Docophoroides murphyi (Kellogg).

Eurymetopus murphyi Kellogg, *Brooklyn Sci. Bull.*, II, iv, pp. 87-89, pl. 16, f. 4, 5 (1914).

(Figs. 32, 33c, 34.)

Two females taken off *Macronectes giganteus* Gm. (giant petrel) at Capetown in November, 1923. This species was described from one male and several females taken off *Thalassarche melanophrys* Boie (mollymawk), *Nealbatrus chlororhynchus* Gm. (yellow-billed mollymawk), and *Macronectes giganteus* in the South Atlantic.

Both sexes of this species can easily be distinguished by the shape of the clypeal signature (Fig. 32), it being much narrower than in the other species, and the females also by the shape of the plates on each side of the meson on sternites ii-v, the median plate on sternites vi to viii (see Figure 34), and by the shape of the lateral plates on the ninth sternite (compare Figures 33 a-d). In *harrisoni* the lateral plates on sternite ii are short, and on sternites iii to v they are elongated. In *brevis* the lateral plates are elongated on sternites ii to iv, and on the fifth sternite there is a long median transverse plate. In both *simplex* and *murphyi* the plates on sternites ii to iv are short and oval in shape, and on sternite v they are slightly longer.

EXPLANATION OF FIGURES.

Fig. 1.—*Lemurphthirus galagus* Bedford, male genitalia.
,, 2.—*Polyplax praomydis* Bedford, head of female.
,, 3.—*Polyplax praomydis* Bedford, pleural plates of female.
,, 4.—*Polyplax praomydis* Bedford, sternal plate.
,, 5.—*Polyplax praomydis* Bedford, male genitalia.
,, 6.—*Bathyergicola hilli* Bedford, head of female.
,, 7.—*Bathyergicola hilli* Bedford, apical sternite of female; (*a*) third pleural plate of same; (*b*) third pleural plate of *Bathyergicola lawrensis* Bedford.
,, 8.—*Bathyergicola hilli* Bedford, male genitalia.
,, 9.—*Bathyergicola lawrensis*, Bedford, head of female.
,, 10.—*Bathyergicola lawrensis* Bedford, apical sternite of female.
,, 11.—*Bucerophagus africanus* Bedford, dorsum and venter of head of female; (*a*) sternal plate.
,, 12.—*Bucerophagus africanus* Bedford, male genitalia.
,, 13.—*Trichodectes cooleyi* Bedford, male.

FIG. 14.—*Tricholipeurus aepycerus* Bedford, male genitalia.

,, 15.—*Tricholipeurus aepycerus* Bedford, head of male.

,, 16.—*Tricholipeurus reduncae* Bedford, head of male.

,, 17.—*Tricholipeurus reduncae* Bedford, male genitalia; (*a*) endomeres of *T. trabeculae* Bedford (drawn to same scale as 17); (*b*) abdominal spiracle of *T. reduncae* Bedford; (*c*) same of *T. trabeculae* Bedford [drawn to same scale as (*b*)].

,, 18.—*Tricholipeurus aepycerus* Bedford, apical sternite of female.

,, 19.—*Tricholipeurus trabeculae* Bedford, apical sternites of female.

,, 20.—*Goniodes scleroptilus* Bedford, male genitalia.

,, 21.—*Goniodes pternistis* Bedford, male genitalia.

,, 22.—*Lipeurus lawrensis* Bedford, head of male; (*a*) sternal plate.

,, 23.—*Lipeurus lawrensis* Bedford, dorsum of abdomen of male; (*a*) apex of abdomen of mounted specimen.

,, 24.—*Lipeurus lawrensis* Bedford, dorsum of abdomen of female.

,, 25.—*Lipeurus pternistis* Bedford, head of female; (*a*) sternal plate.

,, 26.—*Lipeurus pternistis* Bedford, dorsum of abdomen of female.

,, 27.—*Neophilopterus abdimius* Bedford, female.

,, 28.—*Neophilopterus abdimius* Bedford, male genitalia.

,, 29.—*Neophilopterus abdimius* Bedford, apical sternites of female.

,, 30.—*Ibidoecus threskiornis* Bedford, male genitalia.

,, 31.—*Ibidoecus threskiornis* Bedford, apical sternites of female.

,, 32.—*Docophoroides murphyi* Kellogg, forehead of female.

,, 33.—Right lateral plate on ninth sternite of: (*a*) *Docophoroides harrisoni* Waterston; (*b*) *D. simplex* (Waterston); (*c*) *D. murphyi* (Kellogg); (*d*) *D. brevis* (Dufour).

,, 34.—*Docophoroides murphyi* (Kellogg), plates on sternites vi to ix of female.

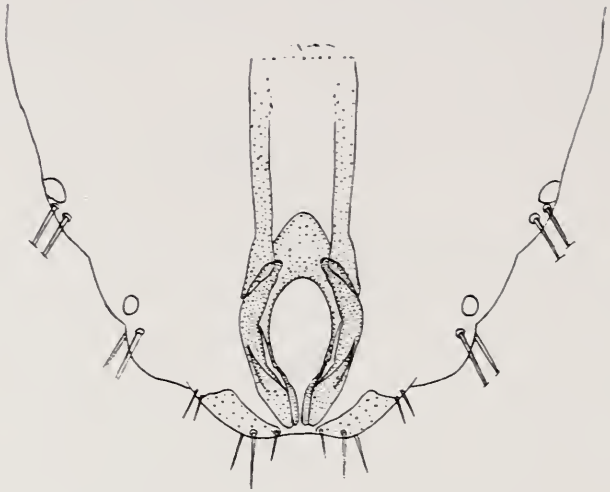


FIG. 1.

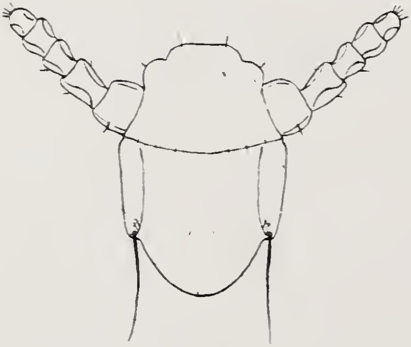


FIG. 2.

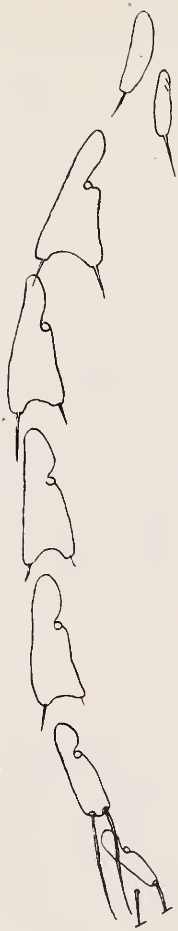


Fig. 3.

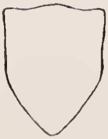


Fig. 4.

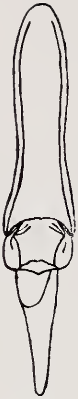


FIG. 5.

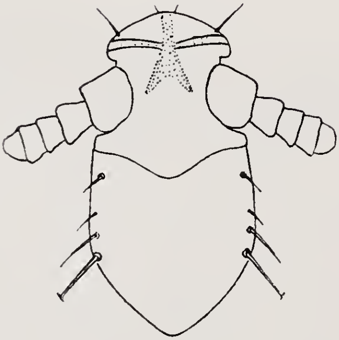


FIG. 6.

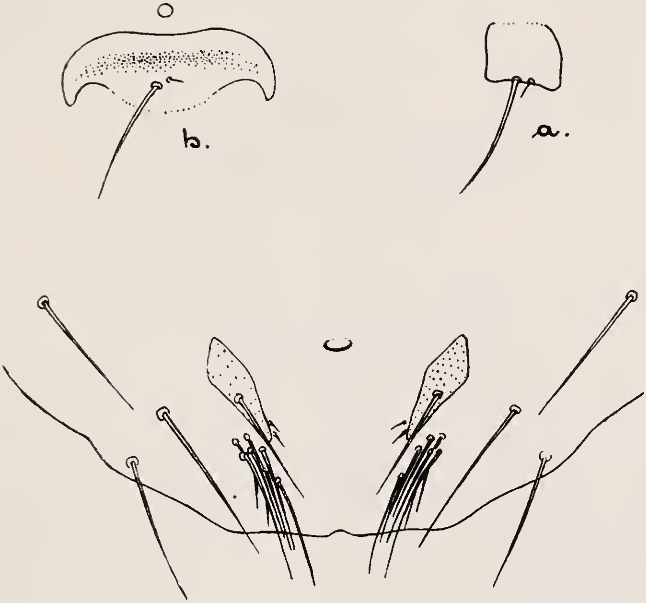


Fig. 7.

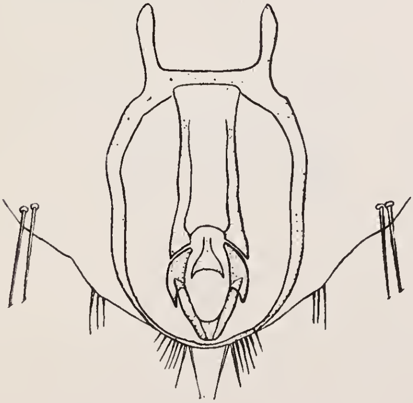


Fig. 8.

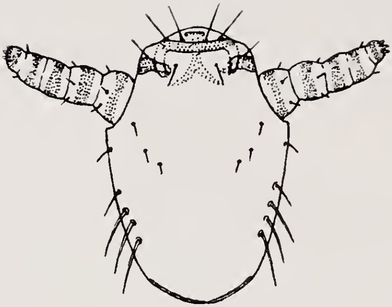


Fig. 9.

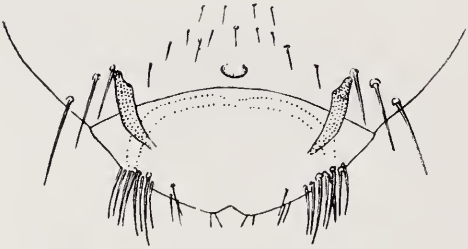


Fig. 10.

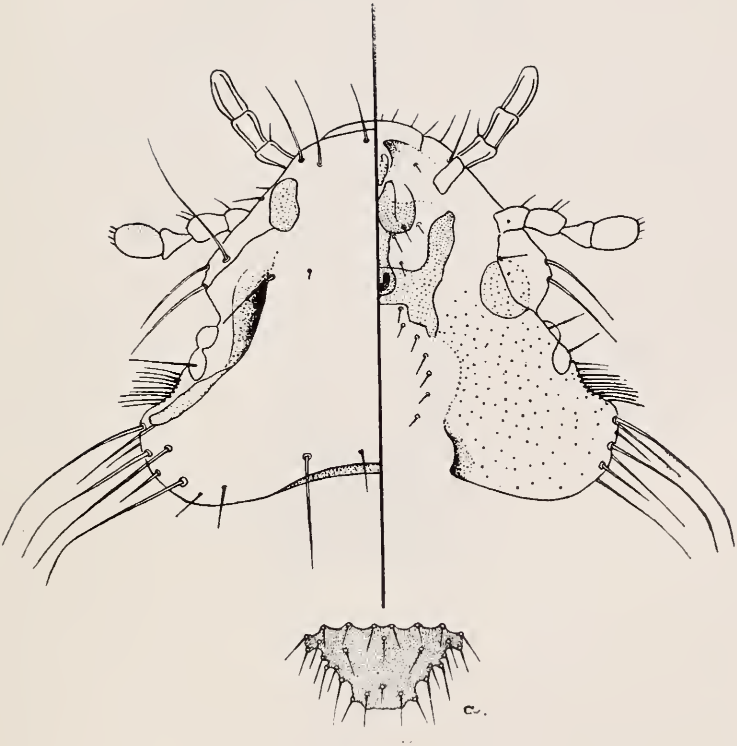


FIG. 11.

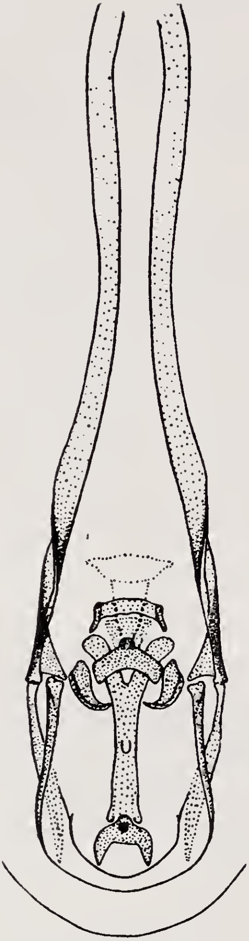


Fig. 12.

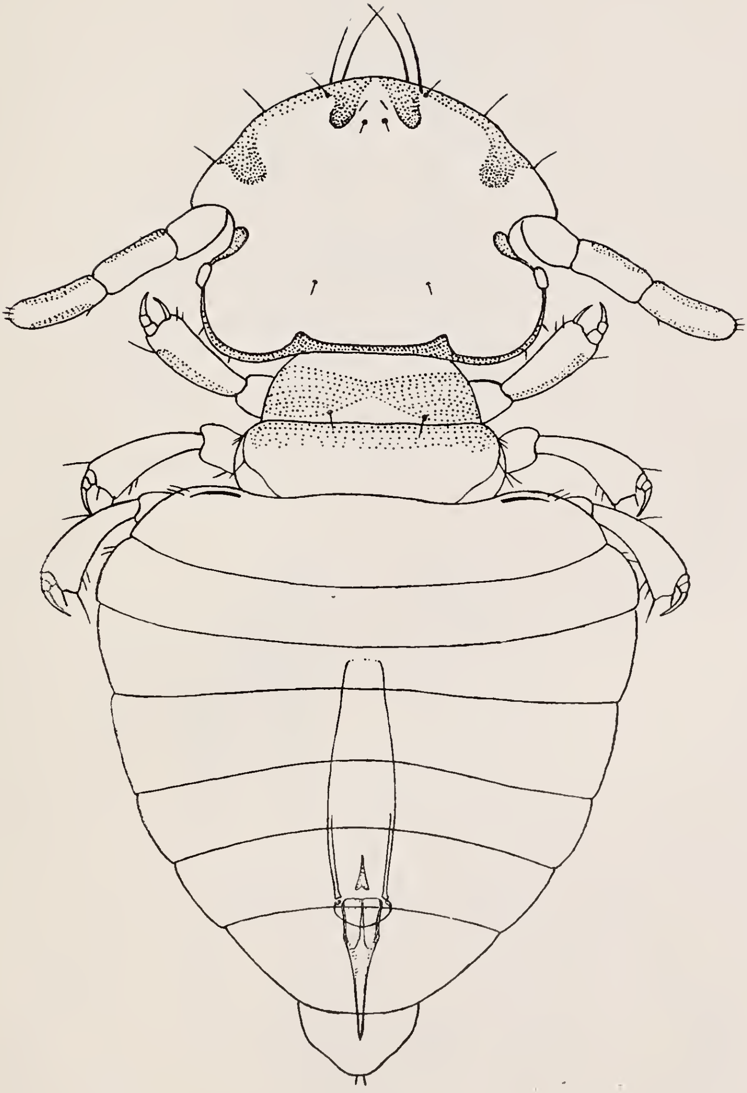


Fig. 13.

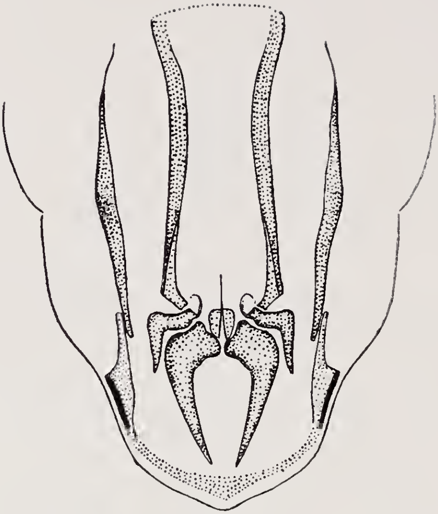


Fig. 14.

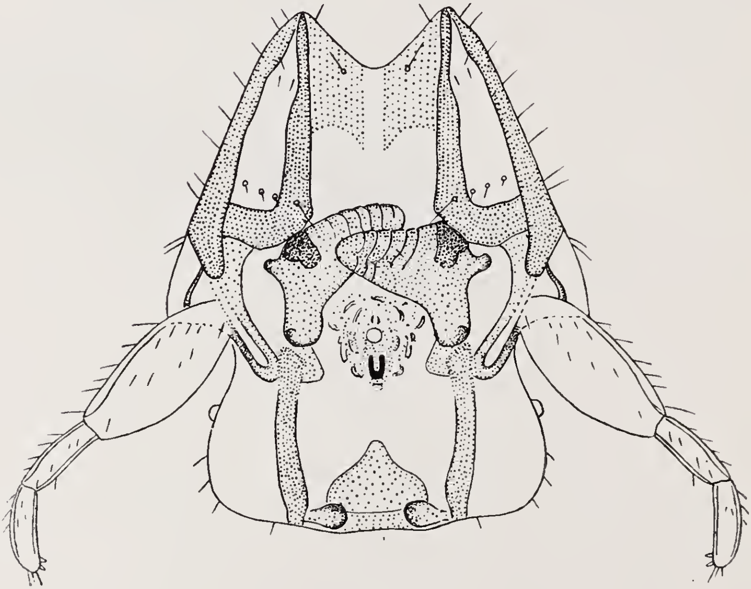


Fig. 15.

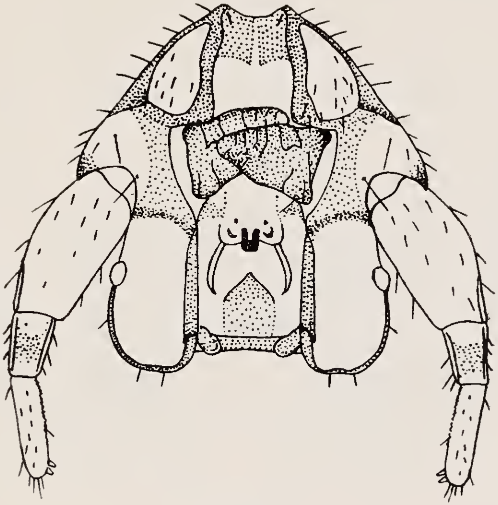


Fig. 16.

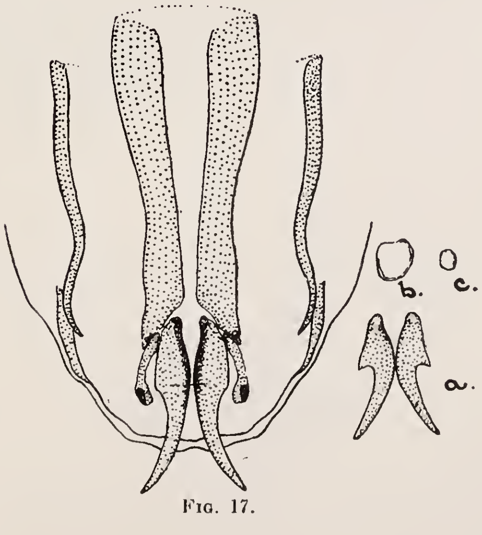


Fig. 17.

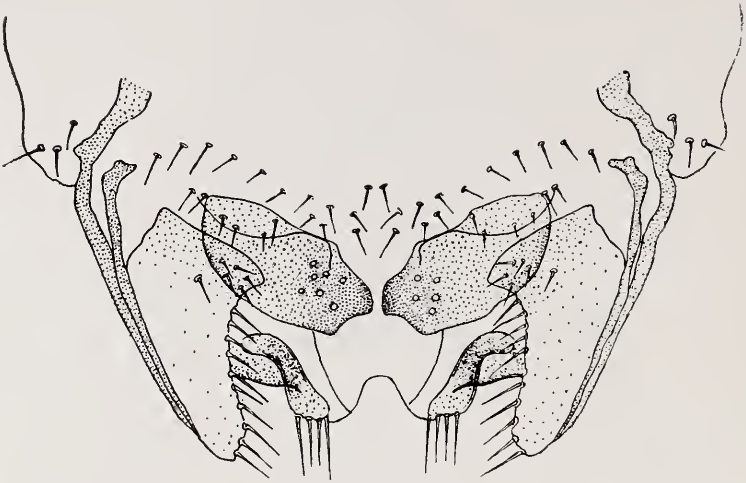


FIG. 18.

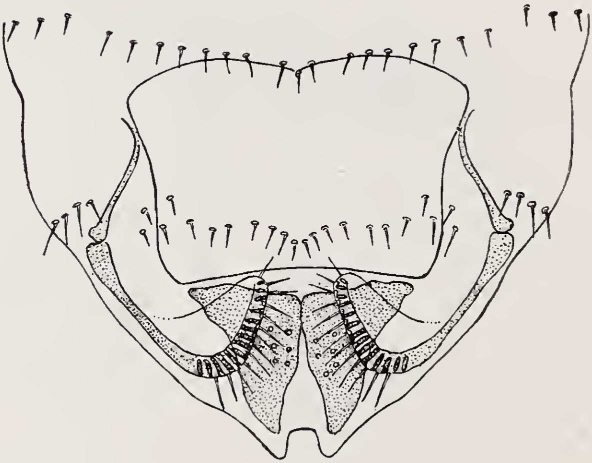


FIG. 19.

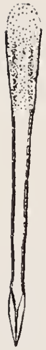


Fig. 20

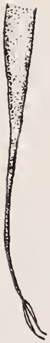
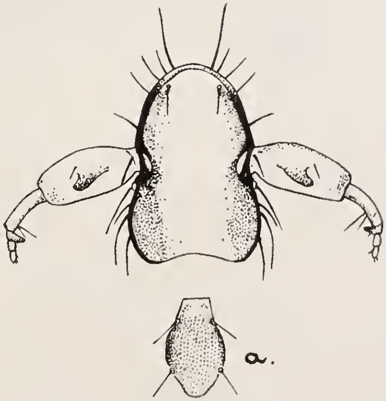


Fig. 21.



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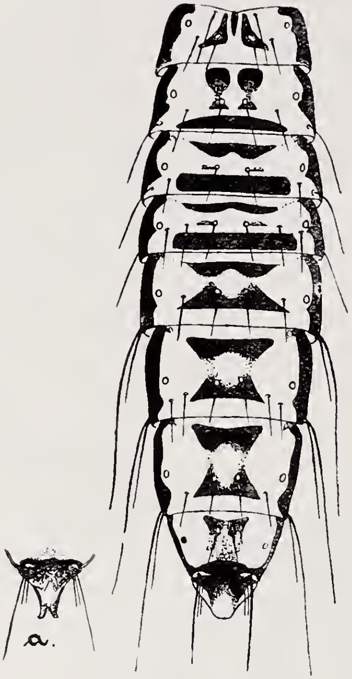

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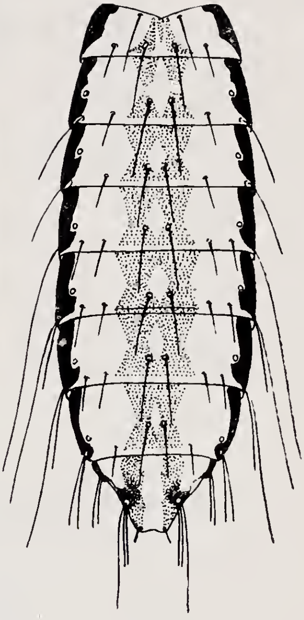

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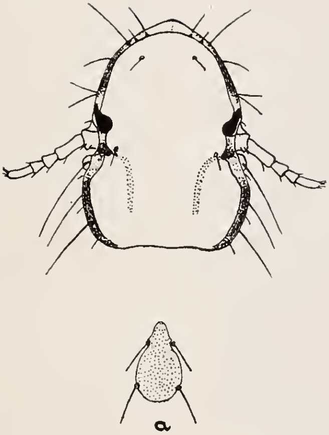


Fig. 25.

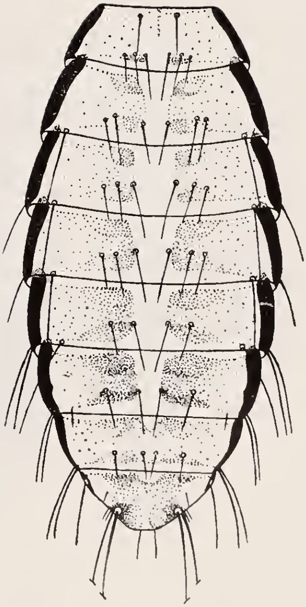


Fig. 26

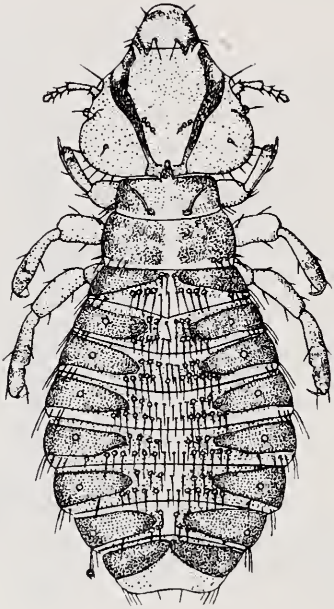


Fig. 27.

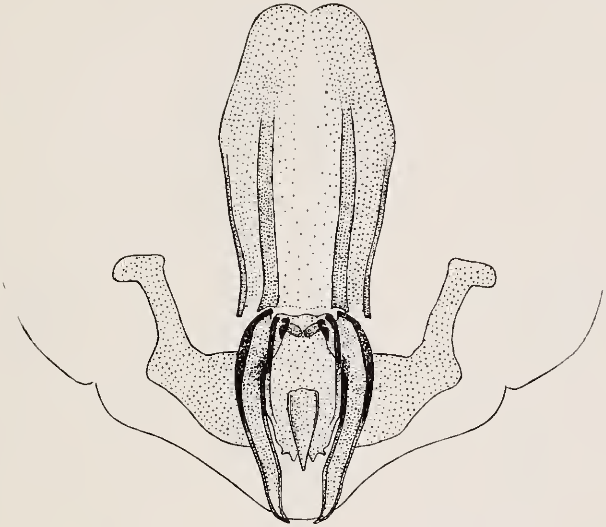
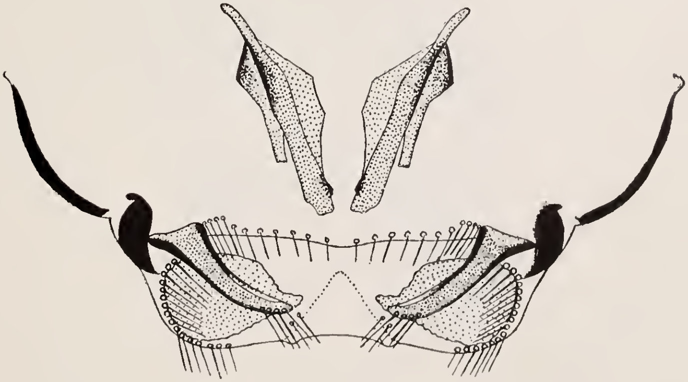


Fig. 28.



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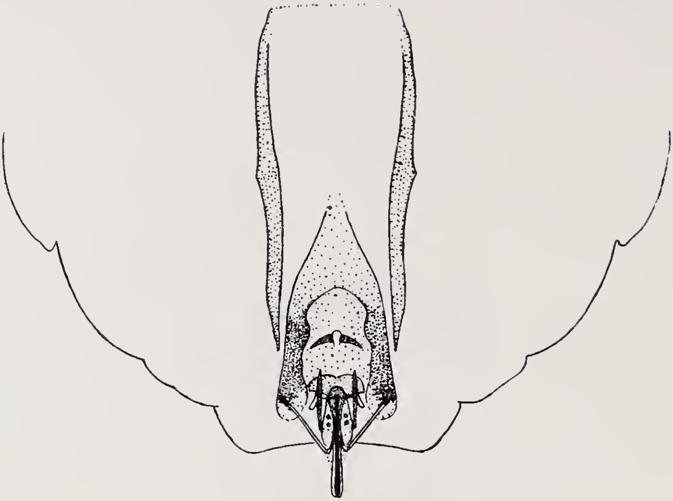

Fig. 30.

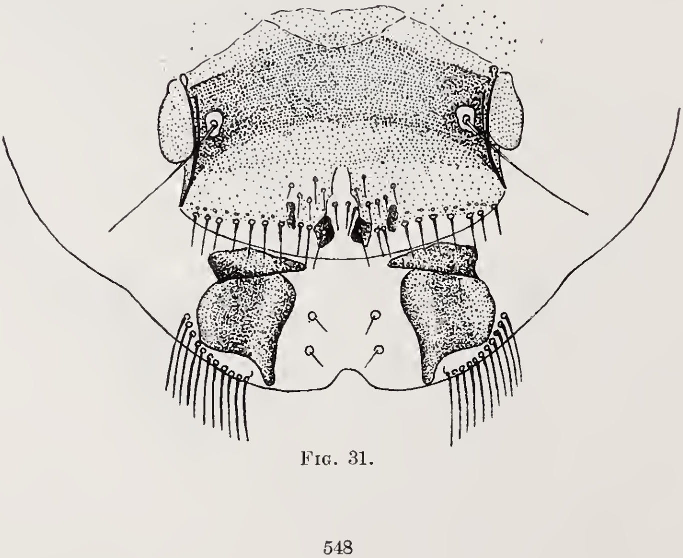

Fig. 31.

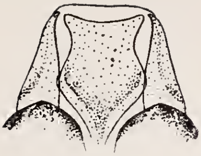

Fig. 32.

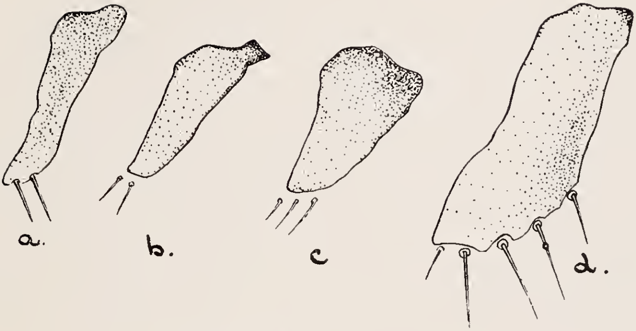

Fig. 33.

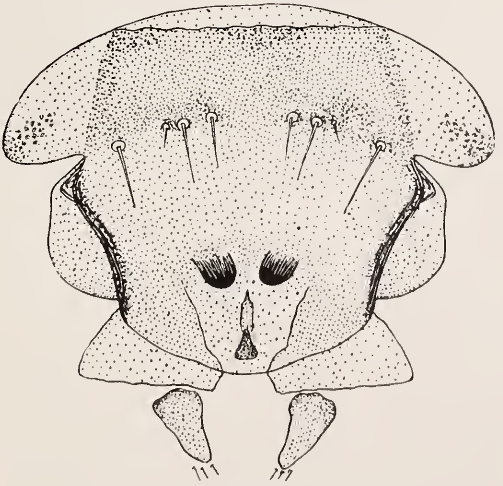

Fig. 34.

15th Annual Report of the Director of Veterinary Services, Union of South Africa. October, 1929.

The Effect upon Ticks of Dipping Cattle regularly at Short Intervals in Arsenical Baths.

By G. A. H. BEDFORD, Research Officer, Onderstepoort.

THE present report is mainly based upon observations and experiments carried out on a grossly infected East Coast fever farm at Sycamore, in the eastern Transvaal, from the end of October, 1927, to the middle of February, 1928.

The farm was taken over by this Division on the 14th September, 1927. At the time there was only one tank on the farm, and all animals from that date were dipped every five days until the 27th October. On this date a new tank had been completed, and it then became possible also to test the 3-day dip and compare the results with those obtained with the 5-day dip.

DIPPING.

For the last 17 or 18 years it has been assumed that the first dipping tank built in South Africa was erected by Mr. Joseph Baynes, C.M.G., on his Nel's Rust Estate in Natal in 1901. However, in 1902, Lounsbury wrote that Mr. Douglass had constructed a dipping vat on his property, Heatherton Towers, in the Cape Province, and carried out some dipping tests, but that various circumstances had led to the abandonment of dipping in favour of spraying with paraffin. Mr. Douglass must have erected his dipping tank prior to 1898, because early in that year he issued an invitation to all and sundry interested in tick eradication to visit his farm on an appointed day to witness a practical demonstration of tick-killing by his new agent. Be that as it may, Mr. Baynes was the first to obtain favourable results with the dipping tank, and his success led to dipping being extensively carried out in South Africa since 1901 with a view to eradicating ticks and eliminating the diseases transmitted by them. Practically all the dipping-fluids that have been used have contained arsenite of soda.

Prior to 1910 the dipping of cattle was carried out at long intervals, intervals of at least ten days, and usually much longer, and, very frequently, at irregular intervals. In 1910, however, Watkins-Pitchford emphasized that a dip capable of application every few days was urgently required to kill the forms of tick life responsible for the spread and perpetuation of East Coast fever, and advocated a dip which could be used every five days without harmful effects to the animals. This dip, known as the 5-day Laboratory Dip, consists of arsenite of soda and water (0.16 per cent. As_2O_3) with the addition of a soap and paraffin emulsion. In the following year he recommended the same dip to be used at a weaker strength (0.08 per cent. As_2O_3) at intervals of three days in order to destroy all larvae and nymphs of *Rhipicephalus appendiculatus* (the chief transmitting agent of East Coast fever), which may develop and drop off their hosts in 72 hours, and even less in certain cases. His contention was that if cattle were dipped regularly every three days no ticks would get on to the animals without being dipped and thereby killed, and should any larvae and nymphs develop in a shorter period than 72 hours, they also would not escape immersion, because he found that regular dipping prevented these immature stages from attaching themselves to their hosts for a considerable period.

It must be stated, however, that in carrying out these tests Pitchford placed larvae and nymphs on the bodies of cattle at various intervals after they had been sprayed, and that these stages do not normally attach themselves to these parts of their hosts.

Watkins-Pitchford found that sodium arsenite alone, used at a strength sufficient to kill the ticks, scalded the cattle. On reducing the amount of arsenic, the dip failed to kill the ticks, but by adding a paraffin and soap emulsion to the more dilute solution, the ticks were destroyed withouh injury to the animals. Cooper and Laws (1915) attributed the increased killing effect of the emulsion to be due to its increased wetting power. The scalding effects which arsenical dips have upon the skins of animals are not solely due to the strength of the arsenite, but also to the climatic and other conditions at the time the animals are dipped.

Within recent years, however, it has been the practice of the majority of farmers in this country to dip their cattle every three, five, or seven days in an arsenical dip without the addition of the paraffin emulsion recommended by Watkins-Pitchford, as it was considered that the arsenite of soda alone was quite as effective, and that the addition of the emulsion did not warrant the extra expense. Furthermore, some gave up using the emulsion, as they considered it was liable to distress the cattle and knock them up, causing sore teats and burning of the skin (Theiler and Gray, 1912). It was, therefore, considered essential that the plain arsenical dip should be thoroughly tested to ascertain whether it was really effective in killing ticks; also whether it is necessary to hand-dress the animals in their ears and under the base of their tails, and to clip the hair on the fringe of the ears and brush of the tails. For these tests the dipped cattle on the farm were divided on the 27th October, 1927, as follows:

A. *Cattle Dipped in 5-Day Dip (Strength* 0.16 *per cent.* As_2O_3).

Lot 1. Animals hand-dressed with tobacco and oil and their ears and tails clipped.
Lot 2. Animals hand-dressed with arsenite and their ears and tails clipped.
Lot 4. Animals not hand-dressed, but their ears and tails clipped.
Lot 5. Animals hand-dressed with tobacco and oil, but not clipped.

B. Cattle Dipped in 3-Day Dip (Strength 0.08 *per cent.* As_2O_3).

Lot 7. Animals hand-dressed with tobacco and oil, and their ears and tails clipped.
Lot 8. Animals hand-dressed with arsenite of soda, and their ears and tails clipped.
Lot 9. Animals not hand-dressed, but ears and tails clipped.
Lot 10. Animals neither hand-dressed nor clipped.
Lot 11. Animals hand-dressed with tobacco and oil, but not clipped.
Lot 12. Animals hand-dressed with arsenite, but not clipped.

It was intended to include animals that were neither hand-dressed nor clipped in lot 3, and animals hand-dressed with arsenite but not clipped in lot 6, but owing to insufficient cattle being available these tests had to be omitted. Animals in lots 1, 2, 5, 7, 8, 11, and 12 had their ears hand-dressed, and they were also hand-dressed under the bases of their tails.

SPECIES OF TICKS FOUND ON CATTLE ON THE FARM.

1. *Rhipicephalus appendiculatus* Neu.—This tick is the chief transmitting agent of East Coast fever. It was the tick most commonly found on the farm, except during October and the early part of November, when both *R. evertsi* and *H. aegyptium impressum* were slightly more numerous, as will be seen by comparing the figures in Table I. When I arrived on the farm this species, *R. appendiculatus*, was rare, as was the case when I paid a short visit to the farm early in October. As will be seen from Chart 1, this species was most numerous during the month of January. During October and November adults were usually only found attached to the ears of cattle, both on the outside and inside; but, later on, when they became more numerous, they were also frequently found on the head, especially on the eyelids, and also occasionally on the neck and around the anus, and less frequently on the body, inside the bases of the legs, fetlocks, and brush of tail. The immature stages were only found inside the ears of the cattle, seated deeper down than the adults, except on two occasions when a nymph was observed attached beneath the base of the tail, and one or two nymphs were found on the outside of the ears.

2. *Rhipicephalus evertsi* Neu.—This tick, known as the "red tick," can also convey East Coast fever. The adults were mainly found under the base of the tails of the cattle, but others were observed on the teats and on the inside of the bases of the legs. It was most numerous during the month of January, but, as will be seen from Chart 1, it was more or less equally prevalent from November to February. The larvae and nymphs were only found in the ears of the cattle, with the exception of one nymph which was seen on the ears of a cow.

3. *Rhipicephalus simus* Koch.—This tick has also been proved to be a transmitting agent of East Coast fever. It was never common on the farm. Adults were occasionally found attached to the brush of the tail and the fetlocks of the cattle, but never on the ears.

4. *Rhipicephalus capensis* Koch.—This species, like the above, can transmit East Coast fever. It was by no means common on the farm. Adults were occasionally found attached to the ears of cattle.

5. *Boophilus decoloratus* Koch.—This species was found on the bodies, necks, and heads of the undipped cattle, and also on the bodies of new arrivals on the farm until they had been dipped regularly for a week or two, but it was never found on cattle that had been regularly dipped for longer periods.

6. *Margaropus winthemi* Karsch.—Several adults and nymphs were found on the body and head of one animal in November. This animal had been on the farm for some time, but was not dipped as it was considered to be immune to East Coast fever. This tick was probably introduced on to the farm with cattle from the Orange Free State.

7. *Hyalomma aegyptium impressum* Neu.—As will be seen from Table II, this species was never common on the farm. In addition to the adults being found under the bases of the tails of the cattle, they were also occasionally found on the underparts, such as the udders of cows and, less frequently, on the brush of the tail and on the legs above the hoofs. The larvae and nymphs attach themselves chiefly to hares, but other small mammals and birds are also attacked.

8. *Amblyomma hebraeum* Koch.—This tick was by no means common on the farm, and was chiefly found on newly imported cattle. The adults attach themselves to the hindquarters beneath the tail of their hosts, also to the teats, testicles, abdomen, and bases of the legs.

TICK INFESTATION ON THE FARM.

During September I paid a short visit to the farm and found that ticks were extremely scarce on the animals, and Mr. White, who was in charge of the stock on the farm, informed me that the ticks were extremely scarce on the animals from the day I visited the farm until I arrived again on the farm at the end of October.

On examining Table I and Chart 1, it will be observed that the ticks continued to be extremely rare on the farm from the end of October until the end of November, and that during this period a large number of animals died of East Coast fever. During December there was a gradual increase of ticks on the farm, and in January the ticks continued to increase in numbers until about the third week, after which they gradually decreased again.

From October until about the end of December the adults of *R. appendiculatus* were only observed on the ears of the dipped cattle, and on these animals only unengorged females were found. During January and the beginning of February, however, adults of this species were also observed on the heads and eyelids of the animals, and, to a much lesser extent, on their bodies and legs. The more numerous the ticks were, the more frequently were they seen attached to these parts, and the more frequently one observed engorged females on the dipped cattle, the latter being mainly found attached to the ears.

It will also be observed, on examining Table I, that the various species of ticks were more numerous on the cattle dipped in the five-day dip than on those dipped in the three-day dip, except on the 30th November, when the average number of *R. appendiculatus* found on the ears of cattle dipped in the three-day dip was 11.9, compared with 10.5, which was the average number found on the ears of cattle dipped in the five-day dip.

TABLES I.—SHOWING THE TICK INFESTATION ON THE FARM AT VARIOUS PERIODS.

Table A.—Adults of *Rhipicephalus appendiculatus* on Ears.

3-Day Dip.				5-Day Dip.			
Date.	No. of Cattle.	No. of Ticks.	Average per Animal.	Date.	No. of Cattle.	No. of Ticks	Average per Animal.
6.11.27	37	25	0·6	10.11.27	27	51	1·8
30.11.27	41	490	11·9	30.11.27	41	433	10·5
21.12.27	43	1,995	46·3	20.12.27	49	2,572	52·4
19. 1.28	12	3,462	288·5	21. 1.28	10	3,970	397·0
18. 2.28	11	474	43·0	17. 2.28	10	719	71·9

Table B.—Ticks under Base of Tail.

Three-day Dip.

Date.	No. of Cattle.	No. of *R. evertsi.*	No. of *R. appendiculatus.*	No. of *H. aegyptium.*	Average per Animal.		
					R. evertsi.	*R. appendiculatus.*	*H. aegyptium.*
6.11.27.....	37	69	—	39	1·8	—	1·0
30.11.27.....	41	129	—	30	3·1	—	0·7
31.11.27.....	43	145	6	41	3·3	0·1	0·9
19. 1.28.....	12	119	44	7	9·9	3·6	0·6
18. 2.28.....	11	32	—	7	2·9	—	0·6

Five-day Dip.

16.11.27.....	27	157	—	33	5·8	—	1·2
30.11.27.....	41	162	—	23	3·9	—	0·5
20.12.27.....	49	254	7	92	5·1	0·1	1·8
21. 1.28.....	10	92	32	16	9·2	3·2	1·6
17. 2.28.....	10	59	4	22	5·9	0·4	2·2

EXPERIMENTAL WORK AND OBSERVATIONS.

1. EFFECTS OF THE 3-DAY AND 5-DAY DIPS UPON ADULTS OF "R. APPENDICULATUS" ATTACHED TO THE EARS, AND UPON THE LARVAE AND NYMPHS OF "RHIPICEPHALUS SPP." FOUND IN THE EARS.

It may be stated that on no occasion was an animal seen to plunge into the dipping tanks without completely immersing its head in the dipping fluids. Therefore, the ears of all the cattle should have been completely wetted at each dipping. In some instances, no live immature ticks were found in the ears of the dipped cattle which had not had their ears hand-dressed; on other occasions one or two live larvae or nymphs were found, and again, in some cases, live immature forms were observed to be numerous. Some of the latter, which were found to be engorged, were collected, with the result that some died, whereas others remained alive and ultimately moulted. These observations prove conclusively that dipping cannot be relied upon to kill the immature stages of ticks in the ears and that it is absolutely essential that the insides of the ears should be hand-dressed.

To test the efficacy of the dips upon the adults of *R. appendiculatus* attached to the ears, all ticks were removed from the ears of cattle either 24 hours previous to dipping or at the time of dipping, and a record was kept of the ticks that attached themselves within 24 hours previous to dipping and up to 48 hours after dipping by marking the positions of the ticks on diagrams. It would have been better to have carried out separate observations on the ticks that attached themselves daily to the ears, but, unfortunately, there were no facilities at the time these observations were made for keeping cattle on tick-free ground. Therefore the animals used had to be sent out daily to graze on the tick-infested veld along with the other cattle, and they frequently became so badly infected with ticks that a large number of observations which were subsequently made thus became unreliable and had consequently to be discarded.

The animals used for these observations which were in the lot that had their ears hand-dressed only had the insides of the ears so treated. Therefore the hand-dressing had no effect upon the adults, except occasionally, when one or two adults attached to the insides of the ears would be lightly smeared with the oil.

Results.—The results of these observations are shown in Table II. It will be observed that both dips killed off a large number of ticks, not only ticks which attached themselves to the ears of animals within 24 hours previous to dipping, but also ticks which attached themselves within 48 hours after dipping. It will be noted that the ticks which attached themselves to the cattle immersed in the five-day dip were more rapidly killed than those found attached to the animals which were immersed in the three-day dip. The results, however, are not as good as one would have wished. Since Watkins-Pitchford found that it was necessary for adult ticks to remain on their hosts for 72 hours in order that they might transmit East Coast fever, these observations also prove that dipping does not prevent a large percentage of the ticks that attach themselves to the ears of cattle from infecting their hosts should such ticks have fed on an infected animal in their nymphal stage. It is essential, therefore, that the whole of the ears should be hand-dressed on an East Coast fever farm. On a non-infected farm, however, only the insides of the ears need be so treated unless adult ticks are numerous on the ears and engorged females are observed.

It will be seen, on examining the tables, that less ticks are recorded as having attached themselves up to 24 hours after dipping than within 24 hours previous to dipping, except that the number of ticks which are recorded as having attached themselves up to 24 hours after dipping to the cattle that were immersed on the 16th January exceeded in numbers those attached before dipping to the cattle immersed in the same dip on the 21st January. For reasons already stated, the figures given, however, do not represent the total numbers of ticks that attached themselves, but it may be stated that the dips do not prevent ticks from attaching themselves to their hosts.

The ticks were sometimes observed to be more numerous on the fringes of the ears than on other parts of these organs, but clipping the fringes did not appear to have any beneficial influence in aiding the dips to kill the ticks.

In order to ascertain the maximum time adults of *R. appendiculatus* remain attached to the ears of cattle that are regularly dipped in a three-day plain arsenical dip, two animals were kept on tick-free ground immediately after they had been dipped on the 9th February, 1928. On the ears of one animal (No. 2169, ears clipped but not hand-dressed) 541 adult ticks were counted when the animal was dipped, 162 being attached to the left ear and 279 to the right ear. Four days later the 45 ticks that were attached to the outsides of the ears were all dead; there were two alive attached to the inside of the left ear, and 94 alive on the inside of the right ear, the majority of these being found attached together in two batches. On the fifth day (second day after second dipping) all the ticks that were attached to the left ear were dead, and there were 22, including two engorged females, alive on the outside of the right ear. On the 9th day there were six partly engorged females alive, and on pulling off one of these they all came away owing to their mouth-parts being stuck together by dried blood. These ticks had survived at least three dippings, probably owing to the fact that they had been unable to feed for several days. The ticks on the ears of the other animal (No. 593, ears neither clipped nor hand-dressed) were too numerous to count when it was dipped on the 9th February, this cow being the worst animal on the farm for picking up ticks. Six days later there were only 26 ticks alive on the ears, and, on the ninth day after the first dipping, ten partly engorged females remained which appeared not to have fed for several days. The ears of this animal were observed to be bleeding badly on the fourth day owing to tick infestation, and the blood on drying may have prevented the ticks from taking in arsenic *per os* when feeding.

TABLES II.—SHOWING THE EFFECT OF ARSENITE OF SODA UPON TICKS.

A.—Ears Infected with Adults of *Rhipicephalus appendiculatus*.

1. *Cattle Dipped regularly in 3-Day Dip (Strength* 0.08 *per cent.).*

All ticks removed on the 9th December, 1927. Animals dipped on the 10th and 13th.

Cattle No.	Ticks Attached day previous to Dipping.				Ticks Attached up to 24 hours after Dipping.			Ticks Attached 24–48 hours after Dipping.	
	10th.	11th.	12th.	13th.	11th.	12th.	13th.	12th.	13th.
2191 (7).....	8	7	5	1	8	8	1	3	0
2577 (7).....	3	3	2	0	4	4	0	1	1
2298 (8).....	6	4	4	1	10	6	4	8	4
2599 (8).....	9	9	7	5	5	4	3	8	3
2206 (9).....	0	—	—	—	—	—	—	12	11
2579 (9).....	4	2	1	0	2	2	0	6	6
565 (10)....	23	21	15	15	12	10	8	29	27
2562 (10)....	20	19	17	12	15	13	10	24	23
1863 (11)....	7	7	7	0	7	5	1	7	1
2117 (11)....	7	7	7	5	4	4	3	0	—
2601 (12)....	15	10	6	2	12	6	3	4	2
2602 (12)....	3	3	2	2	10	10	3	8	8
Total.....	105	92	73	43	89	72	36	110	86
Percentage of Ticks Killed............		12·4	30·5	59		19·1	58·4		21·8

2. *Cattle Dipped regularly in 5-Day Dip (Strength* 0.16 *per cent.).*

All ticks removed on 20th January, 1928. Animals dipped on 21st.

Cattle No.	Ticks Attached Day previous to Dipping.				Ticks Attached up to 24 hours after Dipping.		Remarks.
	21st.	22nd.	23rd.	24th.	22nd.	23rd.	
1781 (1).......	16	7	3	0	—	—	
2560 (1).......	16	10	1	0	—	—	
2565 (2).......	12	3	1	0	—	—	
2233 (4).......	12	9	4	3	—	—	
2235 (4).......	21	18	12	5	3	0	
705 (5).......	31	1	0	0	—	—	
1068 (5).......	23	13	5	5	—	—	
1477..........	10	6	3	1	—	—	Animal not dipped previously.
Total.....	141	67	29	14	3	0	
Percentage of Ticks Killed...........		52·5	79·4	90		100	

TABLES II.—SHOWING THE EFFECT OF ARSENITE OF SODA UPON TICKS—(*continued*).

A.—Ears Infected with Adults of *Rhipicephalus appendiculatus* —(continued).

2. EFFECTS OF THE 3-DAY AND 5-DAY DIPS UPON ADULTS

All ticks removed on the 16th January, 1928. Animals dipped on 16th.

Cattle No.	Ticks Attached up to 24 hours after Dipping				Ticks Attached 24–48 hours after Dipping.			Remarks.
	17th.	18th.	19th.	20th.	18th.	19th.	20th.	
1781 (1)	29	28	9	3	5	0	—	
2560 (1)	28	22	2*	—	2	0	—	* Removed
2120 (2)	9	6	2	0	3	0	—	alive.
2565 (2)	32	29	16	7	0	0	—	
2233 (4)	15	8	1*	—	1	0	—	* Removed
2235 (4)	31	25	21	1	3	3	0	alive.
705 (5)	24	21	10	3	3	2	0	
1068 (5)	6	2	0	—	9	5	2	
2242 (6)	36	35	26	16	5	5	2	
Total...	210	176	87	30	31	15	4	
Percentage of Ticks Killed...		16·2	58·6	85·7		51·6	87	

2. EFFECTS OF THE 3-DAY AND 5-DAY DIPS UPON ADULTS OF "R. APPENDICULATUS" ATTACHED TO THE HEADS OF THEIR HOSTS.

No ticks were found on the heads of the dipped cattle until the adults of *R. appendiculatus* became numerous, when they were observed attached to the eyelids of the animals, and also, to a lesser extent, to other parts. To ascertain how long adults of *R. appendiculatus* remain alive attached to the eyelids of cattle that are dipped regularly in a 3-day plain arsenical dip, two animals were kept on tick-free ground immediately after they had been dipped on the 9th February, 1928. Forty-two adult ticks were counted on the eyelids of these animals when they were dipped. Four days later there were ten alive, the others having been picked off dead; on the fifth day there was only one alive, and this tick was found dead the following day.

The dips had about the same effect upon the ticks attached to these parts as upon those attached to the ears. Therefore, on farms infected with East Coast fever, the ticks on these parts should be smeared with a hand-dressing mixture, and, on non-infected farms, should engorged females be observed, the same procedure should be followed, or the engorged females may be collected by hand. Females of *B. decoloratus* were never found on the heads of the dipped cattle, but were sometimes seen on the heads of the undipped animals.

3. EFFECTS OF THE 3-DAY AND 5-DAY DIPS UPON TICKS ATTACHED TO THE TAIL BRUSHES.

The ticks most commonly found attached to the brushes were adults of *R. simus*, but adults of *H. aegyptium impressum* were also found, and, on very rare occasions, an adult of *R. appendiculatus*. None of these ticks, however, were common on the brushes, and were not found on brushes that had been clipped, nor were they observed on brushes the hair of which was half an inch long, and very rarely on brushes which were three-quarters of an inch long. Why this should have been so it is impossible to explain, because all three species readily attach themselves to other parts of the animals which are either non-hairy or covered with short hairs.

4. EFFECTS OF VARIOUS 3-DAY DIPS UPON TICKS ATTACHED TO THE HINDQUARTERS BENEATH THE TAILS OF CATTLE.

The ticks most common on these parts were adults of *R. evertsi*. Adults of *H. aegyptium impressum* were also found on them, and also occasionally adults of *R. appendiculatus* when this tick was numerous. The adults of *A. hebraeum* also attach themselves to these parts, but this species was very rarely seen on animals at Sycamore and was by no means common on cattle used for carrying out dipping tests at Onderstepoort. It is generally contended that the parts beneath the bases of the tails of animals are not wetted when the animals plunge into and swim through the tanks, and that in order to kill the ticks attached to these parts it is necessary to hand-dress. However, the following tests that were carried out both at Sycamore and Onderstepoort prove that these parts are wetted, and that dipping is quite as effective as spraying. Moreover, a large percentage of ticks that attached themselves to these parts up to 48 hours after dipping in a 3-day dip and up to 72 hours after immersion in a 5-day dip were killed as a result of dipping.

To ascertain the effect of dipping upon ticks attached to the bases of the tails of cattle, all ticks were removed from these parts of animals that were regularly dipped at Sycamore, and records kept of the ticks that afterwards became daily attached, by marking the positions of the ticks on diagrams. The results of these tests are shown in Tables III.

Further tests were subsequently undertaken at Onderstepoort, where both *R. evertsi* and *H. aegyptium impressum* are more common, to confirm the above observations, and also to compare the killing effects of various dipping fluids upon ticks.

TABLES III.—SHOWING THE EFFECT OF ARSENITE OF SODA UPON TICKS.

A.—Animals Infected with Adults of *Rhipicephalus evertsi*, *R. appendiculatus*, and *Hyalomma aegyptuim impressum* under Tail.

1. *Cattle Dipped regularly in 3-Day Dip (Strength* 0.08 *per cent.).*

No. of cattle 12. All ticks removed on 9th December, 1927. Animals dipped on 10th.

	Ticks Attached Day previous to Dipping.				Ticks Attached up to 24 hours after Dipping.			Ticks Attached 24–48 hours after Dipping.	
	10th.	11th.	12th.	13th.	11th.	12th.	13th.	12th.	13th.
R. evertsi....	10	10	3	2	12	8	0	12	6
H. aegyptium	3	3	3	2	2	1	0	2	0

No. of cattle 11. All ticks removed on 14th December, 1927. Animals dipped on 13th, 16th, and 19th.

	Ticks Attached 24–48 hours after Dipping.					Ticks Attached Day previous to Dipping.				
	15th.	16th.	17th.	18th.	19th.	16th.	17th.	18th.	19th.	20th.
R. evertsi.......	9	5	3	3	0	4	4	1	1	0
H. aegyptium...	6	5	3	2	0	1	1	1	1	1

Cow No. 2433.—All ticks removed on 30th January, 1928. Dipped on 30th January and 2nd February.

	Tick Attached 24–48 hours after Dipping.				Tick Attached up to 24 hours after Dipping.	
	1st.	2nd.	3rd.	4th.	3rd.	4th.
R. evertsi.........	1	1	1	0	1	0

2. *Cattle Dipped regularly in 5-Day Dip (Strength* 0.16 *per cent.).*

No. of cattle 8. All ticks removed on 20th January, 1928. Animals dipped on 21st.

	Ticks Attached Day previous to Dipping.			
	21st.	22nd.	23rd.	24th.
R. evertsi.....................	4	1	1	0
R. appendiculatus...............	2	1	0	—

No. of cattle 10. All ticks removed on 16th January, 1928. Animals dipped on the 16th.

	Ticks Attached up to 24 hours after Dipping.			
	17th.	18th.	19th.	20th.
R. evertsi.....................	6	5	2	0

No. of cattle 2. All ticks removed on the 2nd February, 1928. Animals dipped on 31st January.

	Ticks Attached 48–72 hours after Dipping.		
	3rd.	4th.	5th.
R. evertsi......................	8	8	1
H. aegyptium..................	1	1	0

TABLES IV.—SHOWING THE EFFECT OF VARIOUS DIPPING FLUIDS UPON TICKS.

1. CATTLE SPRAYED UNDER TAIL WITH ARSENITE OF SODA (STRENGTH 0.16 PER CENT. As_2O_3).

(*a*) Rhipicephalus evertsi *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
* 4. 9.28	3690	102	—	—	—	—	—
24. 9.28	4532	63	12	4	3	3	—
16.10.28	4532	93	18	7	4	—	—
22.10.28	3701	155	7	5	—	—	—
29.10.28	4558	172	5	4	3	(1 eng. ♀)	—
			(1 eng. ♀)				
5.11.28	3701	237	9	7	4	4	4
26.11.28	4532	67	8	2	1	1	—
				(1 eng. ♀)			
26.11.28	4558	110	5	1	1	—	—
† 4.12.28	3690	182	3	—	—	—	—
† 4.12.28	3701	167	—	—	—	—	—
TOTAL.		1,348	67	30	16	9	4
Percentage of Ticks Killed..			95	97·7	98·8	99·3	99·7

* Animal sprayed for first time.
† Sprayed lightly without jet.

(*b*) Hyalomma aegyptium impressum *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
* 4. 9.28	3690	10	2	1	1	—	—
24. 9.28	4532	1	1	1	1	—	—
16.10.28	4532	5	1	—	—	—	—
22.10.28	3701	2	1	1	—	—	—
29.10.28	4558	11	7	3	3	(1 eng. ♀)	—
			(1 eng. ♀)		(1 eng. ♀)		
5.11.28	3701	12	1	—	—	—	—
26.11.28	4532	16	5	3	3	(1 eng. ♀)	
26.11.28	4558	14	7	2	2	2	1
			(1 eng. ♀)				
† 4.12.28	3690	20	—	—	—	—	—
† 4.12.28	3701	30	—	—	—	—	—
TOTAL.		121	25	11	10	4	1
Percentage of Ticks Killed..			79·3	90·9	91·7	96·7	99·2

* Animal sprayed for first time.
† Sprayed lightly without jet.

TABLES IV.—SHOWING THE EFFECT OF VARIOUS DIPPING FLUIDS UPON TICKS—(*continued*).

2. CATTLE SPRAYED UNDER TAIL WITH FIVE- TO SEVEN-DAY LABORATORY DIP (ARSENITE OF SODA, STRENGTH 0.16 PER CENT., PLUS SOAP AND PARAFFIN).

(*a*) Rhipicephalus evertsi *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
5.2.29	4532	69	3 (1 eng. ♀)	1	0	—	—
5.2.29	3701	67	—	—	—	—	—
11.2.29	4558	80	—	—	—	—	—
11.2.29	3690	99	5	—	—	—	—
18.2.29	4532	66	2	2	2	1	1
18.2.29	3701	101	—	—	—	—	—
25.2.29	4558	113	12	6 (1 eng. ♀)	1	—	—
25.2.29	3690	158	11	5	—	—	—
4.3.29	4532	41	6	2	1	1	1
4.3.29	3701	109	1	—	—	—	—
TOTAL.	....	903	40	16	4	2	2
Percentage of Ticks Killed..			95·5	98·2	99·5	99·7	99·7

(*b*) Hyalomma aegyptium impressum *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying				
			1.	2.	3.	4.	5.
5.2.29	4532	30	22 (1 eng ♀)	9	2	—	—
5.2.29	3701	7	—	—	—	—	—
11.2.29	4558	—	—	—	—	—	—
11.2.29	3690	—	—	—	—	—	—
18.2.29	4532	17	5	3	1	1	1
18.2.29	3701	6	—	—	—	—	—
25.2.29	4558	2	1	1	—	—	—
25.2.29	3690	2	—	—	—	—	—
4.3.29	4532	6	1	—	—	—	—
4.3.29	3701	9	3	—	—	—	—
TOTAL.	....	79	32	13	3	1	1
Percentage of Ticks Killed..			60	83·5	96·2	98·7	98·7

TABLES IV.—SHOWING THE EFFECT OF VARIOUS DIPPING FLUIDS UPON TICKS—(*continued*).

3. CATTLE SPRAYED UNDER TAIL WITH COPPER SULPHATE DIP ($CuSO_4$ 8 LB., As_2O_3 2 LB., HCL 1.6 PINTS, WATER 100 GALLS.).

(*a*) Rhipicephalus evertsi *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
21.1.29	3701	91	4 (1 eng. ♀)	1	—	—	—
21.1.29	4532	64	19	1	1	—	—
12.3.29	4558	75	20	4	2	2	1
12.3.29	3690	84	17	9	6	6	3
18.3.29	3701	94	8	1	1	—	—
*18.3.29	4532	62	16	8	2	1	1
*18.3.29	2782	39	8	2	1	1	1†
18.3.29	2785	112	21	2	1	1	1
25.3.29	2786	125	10	6	6	6	6
25.3.29	2787	102	10	4	1 (1 eng. ♀)	—	—
TOTAL........		848	133	38	21	17	13
Percentage of Ticks Killed..			84·3	95·5	97·5	97·7	98·4

* Animal sprayed for first time.
† ♀ very slightly engorged.

(*b*) Hyalomma aegyptium impressum *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
21.1.29	3701	15	2	—	—	—	—
21.1.29	4532	7	1	—	—	—	—
12.3.29	4558	2	1	1	1	1	—
12.3.29	3690	—	—	—	—	—	—
18.3.29	3701	2	1	—	—	—	—
18.3.29	4532	9	5	4	3	3	3*
†18.3.29	2782	2	1	—	—	—	—
†18.3.29	2785	3	1	—	—	—	—
25.3.29	2786	5	—	—	—	—	—
25.3.29	2787	4	—	—	—	—	—
TOTAL........		49	12	5	4	4	3
Percentage of Ticks Killed..			75·7	89·7	91·8	91·8	93·8

* 1 engorged ♀, 1 unengorged ♀, and 1 ♂.
† Animal sprayed for first time.

TABLES IV.—SHOWING THE EFFECT OF VARIOUS DIPPING FLUIDS UPON TICKS—(*continued*).

4. Cattle Sprayed under Tail with Cooper's Improved Cattle Dip (Strength 0.16 per cent. As_2O_3).

(*a*) Rhipicephalus evertsi *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
* 4. 9.28	3701	96	20	—	—	—	—
*10. 9.28	4532	79	36	2	—	—	—
24. 9.28	4558	101	38	5	4 (2 eng. ♀)	—	—
22.10.28	3690	184	9	8	5	4	3
12.11.28	4558	127	14 (2 eng. ♀)	2	1	1	1
12.11.28	4532	179	9	3	2	1	1*
19.11.28	3690	174	—	—	—	—	—
19.11.28	3701	185	3	3	—	—	—
‡10.12.28	4532	242	19 (1 eng. ♀)	10 (4 eng. ♀)	5	2	2
‡10.12.28	4558	162	7 (4 eng. ♀)	1	—	—	—
Total........		1,529	155	34	17	8	7
Percentage of Ticks Killed..			89·9	97·8	98·9	99·5	99·5

* Animal sprayed for first time.
† Tick in fold of skin, and probably escaped spraying.
‡ Sprayed lightly with jet.

(*b*) Hyalomma aegyptium impressum *under Base of Tail.*

Date of Spraying.	Cattle No.	No. of Ticks Day of Spraying.	Days after Spraying.				
			1.	2.	3.	4.	5.
* 4. 9.28	3701	5	3	2	—	—	—
*10. 9.28	4532	8	8	2	—	—	—
24. 9.28	4558	6	3	2	2	—	—
22.10.28	3690	3	1	—	—	—	—
12.11.28	4558	8	2	2	2	2	1
12.11.28	4532	23	16 (1 eng. ♀)	9 (2 eng. ♀)	7	6	6† (1 eng. ♀)
19.11.28	3690	2	1	1	—	—	—
19.11.28	3701	3	—	—	—	—	—
‡10.12.28	4532	25	10	3	2 (1 eng. ♀)	—	—
‡10.12.28	4558	15	2	(1 eng. ♀)	—	—	—
Total........		98	46	22	13	8	7
Percentage of Ticks Killed..			53·1	77·5	86·7	91·8	92·8

* Animal sprayed for first time.
† Ticks in fold of skin, and probably escaped spraying.
‡ Sprayed lightly without jet.

TABLES IV.—SHOWING THE EFFECT OF VARIOUS DIPPING FLUIDS UPON TICKS—(*continued*).

5. Cattle Dipped in Cooper's Improved Cattle Dip (Strength 0.16 per cent. As_2O_3).

(*a*) Rhipicephalus evertsi *under Base of Tail.*

Date of Dipping.	Cattle No.	No. of Ticks Day of Dipping.	Days after Dipping.				
			1.	2.	3.	4.	5.
*10. 9.28	4558	77	56	10	3	(1 eng. ♀)	—
18.12.28	3690	185	—	—	—	—	—
18.12.28	3701	158	—	—	—	—	—
14. 1.29	3690	147	—	—	—	—	—
14. 1.29	4558	128	8	—	—	—	—
Total.	....	695	64	10	3	1	—
Percentage of Ticks Killed..			90·8	98·5	99·5	99·9	100·0

* Animal dipped for first time.

(*b*) Hyalomma aegyptium impressum *under Base of Tail.*

Date of Dipping.	Cattle No.	No. of Ticks Day of Dipping.	Days after Dipping.				
			1.	2.	3.	4.	5.
*10. 9.28	4558	10	7	6	1	1	1
18.12.28	3690	15	2 (1 eng. ♀)	(1 eng. ♀)	—	—	—
18.12.28	3701	24	—	—	—	—	—
14. 1.29	3690	13	—	—	—	—	—
14. 1.29	4558	26	6 (3 eng. ♀)	—	—	—	—
Total.	....	88	15	7	1	1	1
Percentage of Ticks Killed..			83	92	99	99	99

* Animal dipped for first time.

The cattle used for these tests were sent to a tick-infested farm where they remained for a week. Then they were brought to the Laboratory, the ticks attached beneath their tails were counted, and the animals either sprayed with a hand-sprayer (bucket type) or dipped. After spraying or dipping, the animals were kept in a tick-free stable, and every day they were examined, the ticks counted, and the dead ones removed. At the end of a week they were sent back to the farm, and other animals brought in for further tests.

In comparing the efficacy of various dipping fluids, spraying was adopted in preference to dipping, not only because the results of spraying were found to be as reliable as dipping, and the results obtained with both methods were practically the same (compare Tables IV, 4 and 5), but also because spraying considerably reduced the cost of the tests and saved a considerable amount of labour. For spraying the animals under the tails less than 4 gallons of fluid was required, whereas had the animals been dipped it would have been necessary to have made up 4,000 gallons of fluid for each dip tested.

The results obtained are shown in Tables IV, except that, in addition to these, eleven adults of *A. hebraeum* were observed on cattle sprayed with Cooper's Improved Cattle Dip and plain As_2O_3. Of these, six were found to be dead the day after spraying, and on the following day all had died. On examining these tables, it will be observed that on the whole excellent results were obtained, and that the results obtained with the plain arsenical 5-day dip were either practically or equally as good as the results obtained with the same fluid containing various additional chemicals. The addition of these chemicals, therefore, does not warrant their extra cost. The arsenical dip containing copper sulphate was tested because, some years ago, we undertook a large number of dipping tests with various chemicals by immersing engorged females of *B. decoloratus* in them *in vitro*, and this preparation gave the most promising results. In addition to the dipping fluids listed in the tables, similar spraying tests were also carried out with "Volck," a preparation made by the California Spray Chemical Co., and a Mexican marigold solution, made by boiling 1 lb. of the plant in 4 gallons water. These fluids had no ill-effect upon the ticks, and therefore acted as controls to the above tests.

5. EFFECTS OF THE 3-DAY AND 5-DAY PLAIN ARSENICAL DIPS UPON TICKS ATTACHED TO THE BODIES AND LEGS OF ANIMALS.

The ticks attached to the bodies and legs of dipped cattle at Sycamore were not nearly as numerous as those observed on these parts on the undipped cattle, and engorged females were very rarely seen on them except when the ticks became very numerous, and then only occasionally. Females of *B. decoloratus* were never observed on the bodies and legs of cattle that had been regularly dipped for more than two weeks, although they were fairly numerous on these parts on undipped cattle. Although no observations were made to ascertain the mortality rate of the ticks attached to these parts as a result of dipping, it may be stated that the effects of the dips upon ticks attached to these parts were even more favourable than with those ticks attached to the hindquarters beneath the tails.

6. EFFECTS OF ARSENICAL DIPS UPON FEMALES THAT LIVE TO ENGORGE THEMSELVES UPON DIPPED CATTLE.

Fully engorged females were collected from various parts of dipped cattle, and the majority of these either died within a few days without laying eggs, or laid an abnormally small number of eggs that failed to hatch. Very rarely did a female lay eggs that retained their vitality and hatched in due course.

SUMMARY AND CONCLUSIONS.

Effects of Various Dipping Fluids upon Adult Ticks that attach Themselves to Animals that are Regularly Dipped.

In South Africa there are a large number of farmers who grossly underestimate the value of dipping, and some have even contended that while dipping keeps ticks in check for a time, after a period of regular dipping the fluids lose their killing powers, and this they attribute to the fact that the ticks gradually become resistant to arsenical poisoning in the same way as animals acquire tolerance to a certain extent. Their reasoning, although incorrect, is easy to understand. Had the dipping tests at Sycamore been carried out by a farmer, he would have observed that the more frequently he dipped the animals the more badly infected with ticks they became, and he would undoubtedly have come to the conclusion that the dipping fluids used were worse than useless. Such, however, was far from being the case. Farmers are not in a position to judge the efficacy of a dip by comparing from time to time the number of ticks attached to animals that are regularly dipped, because there is no dipping fluid known that will prevent ticks getting on to animals, and the number of ticks seen on animals will vary in different seasons of the year owing to climatic conditions and the life-cycles of the ticks. Also, the tick infestation may vary weekly if animals are grazed on different parts of a farm. Moreover, on examining animals, the same species of ticks are invariably found attached to the same parts of their hosts, and this will mislead the farmer into believing that he is always seeing the same individual ticks week after week. The only species of ticks which should not be found on animals after they have been regularly dipped for two or more weeks in either a 3-, 5-, or 7-day dip are *Boophilus decoloratus* and *Margaropus winthemi*. This is because these species are one-host ticks, and the immature stages which are too small to be observed are killed by the dipping fluids before the stages become adults.

The experiments carried out both at Sycamore and Onderstepoort prove that:—

1. The plain arsenical dip used at both 3- and 5-day strengths, also the Laboratory and copper sulphate dips used at 5-day strengths, and Cooper's Improved Cattle Dip at the strength recommended by the makers for use at 14 days' interval (at this strength it can also be used at 5-7 days' interval) can be relied upon to kill a very large percentage of ticks that attach themselves to animals that are

regularly dipped. Furthermore, should females live sufficiently long on dipped animals to engorge themselves, the majority either die within a few days after leaving their hosts without laying eggs, or, should they lay, the eggs fail to hatch. Very rarely does a female that drops off a dipped animal lay eggs that retain their vitality.

2. The results of the spraying tests (shown in Tables IV), undertaken to compare the killing effects of the above arsenical dips upon ticks, showed that the plain arsenical 5-day dip (0.16 per cent. As_2O_3) was either practically or equally as good as the same fluid containing various additional chemicals, and that the addition of these chemicals, therefore, does not warrant their extra cost.
3. The dips do not prevent the ticks from attaching themselves to their hosts after the latter have been immersed.
4. The dipping fluids not only kill a very large percentage of the ticks that are attached when their hosts are dipped, but it was also observed that the percentage of deaths invariably increased in the case of ticks that attached themselves to the ears of cattle up to 48 hours after the animals were immersed in either the 3-day (0.08 per cent. As_2O_3) or 5-day (0.16 per cent. As_2O_3) plain arsenical dips, and also in the case of ticks that attached themselves to the hindquarters beneath the tails of cattle up to 48 hours after their hosts had been dipped in a 3-day dip, and up to 72 hours after their hosts had been immersed in a 5-day dip.
5. Dipping is far more effective in killing ticks attached to the hindquarters beneath the tails of their hosts than is generally considered to be the case; the results obtained by dipping being equally as good as those obtained by spraying ticks attached to these parts with the same fluid used at the same strength.
6. The dips are more effective in killing adult ticks attached to the bodies and beneath the tails of their hosts than those attached to the ears and heads. Only 52.5 per cent. of the adults of *R. appendiculatus* that were attached to the ears of cattle that had been dipped in a 0.16 per cent. As_2O_3 solution died within 24 hours after dipping; 79.4 per cent. of the ticks were dead two days after dipping, and 90 per cent. were dead on the third day after dipping. Of adults of *R. evertsi* attached beneath the bases of the tails of cattle sprayed with the same solution, 95 per cent. were found to be dead 24 hours after spraying.
7. Clipping the hair on the fringe of the ears appeared to have no beneficial effect in killing the ticks.
8. Since the larvae and nymphs normally only live in the ears of their hosts, they can be satisfactorily destroyed by means of a suitable hand-dressing mixture applied every three days. Should these stages attach themselves to other parts of the body, regular dipping at five days' interval can be relied upon to kill them, and any animals that cannot be dipped should be sprayed with a hand-pump.

9. Regular dipping of stock should be carried out every five days on farms infected with East Coast fever, and every five to seven days on non-infected farms. Dipping cannot be relied upon to kill adult ticks within three days, the time required for infected ticks to remain on their hosts to infect them. However, the majority of the adults of *R. appendiculatus* attach themselves to the ears of their hosts, and can be speedily destroyed by applying a suitable hand-dressing mixture. Ticks attached to other parts of the head should be dealt with in a similar manner. The adults of *R. evertsi* mainly attach themselves to the hind-quarters beneath the tail and can likewise be dealt with by hand-dressing. Those of *R. simus* attach themselves to the fetlocks and brushes of the tails of cattle, and the latter can be kept free of ticks by clipping.

10. In the past it has frequently been advocated that an arsenical dip containing 0.24 per cent. As_2O_3 should be used at a 14-day interval to eradicate *Amblyomma hebraeum*, the transmitting agent of heartwater, because it was considered that weaker solutions used at shorter intervals were ineffective against this species. It must be admitted that the stronger the dip the more effective it will be in killing ticks, but as the 14-day interval is too long a period on account of the short time the ticks remain upon their hosts, the 7-day strength used at five to seven days' interval is bound to be more effective, especially in destroying the immature stages of ticks attached to various parts of animals other than the inside of the ears. In Australia, farmers have been using the 14-day dip to eradicate *Boophilus microplus* (*australis*), and Sir Arnold Theiler informs me that this dip was by no means effective in killing this species. If the 14-day dip is not effective against species of *Boophilus*—which are reputed to be the easiest ticks to kill owing to their having a very short proboscis, and, moreover, being one-host ticks, remain on their hosts for longer periods than other species—then surely this dip cannot possibly be effective against other species of ticks. Both the 3-day and 7-day dips are very effective in killing species of *Boophilus*, therefore they are bound to be more effective than the 14-day dip in killing other species of ticks. Furthermore, the results of tests carried out with 7-day dips proved that this dip is very effective in killing adults of *R. evertsi* and *H. aegyptium*, the latter being as difficult to kill as *A. hebraeum*, and the few observations we were able to make on the effects of this dip upon adults of *A. hebraeum* were also entirely satisfactory.

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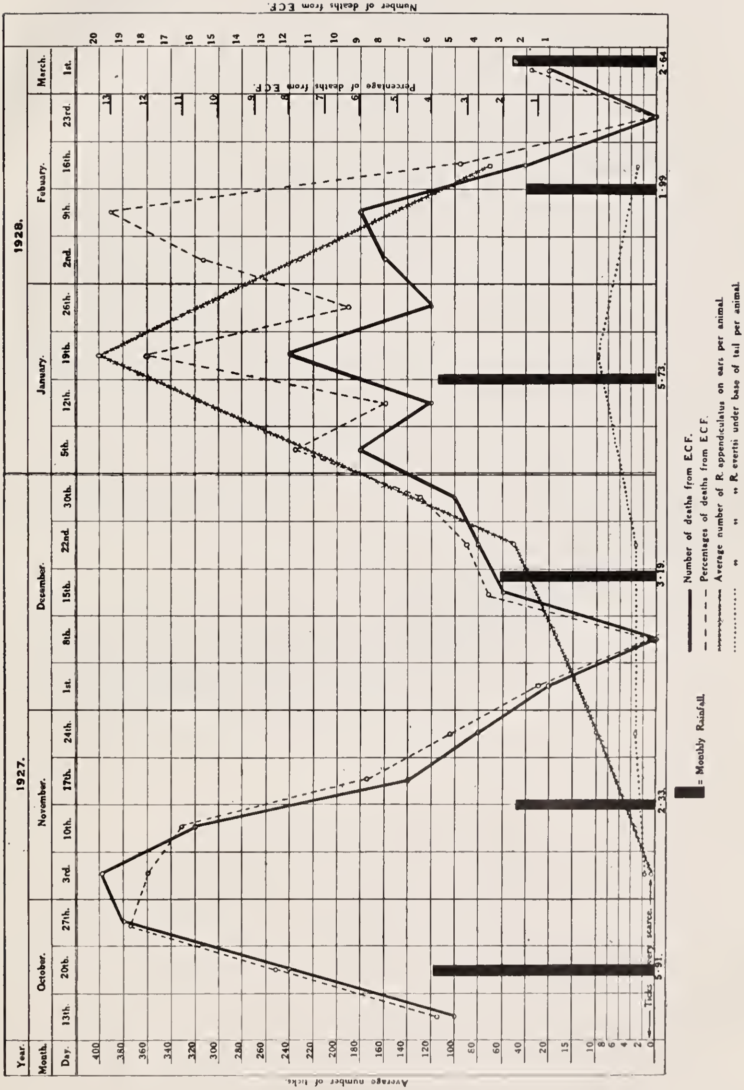
Year.
Month.
Day.
1927.
1928.
October.
November.
December.
January.
Febuary.
March.
13th.
20th.
27th.
3rd.
10th.
17th.
24th.
1st.
8th.
15th.
22nd.
30th.
5th.
12th.
19th.
26th.
2nd.
9th.
16th.
23rd.
1st.
Average number of ticks.
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
15
10
8
6
4
2
0
Number of deaths from E.C.F.
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Percentage of deaths from E.C.F.
13
12
11
10
9
8
7
6
5
4
3
2
1
Ticks very scarce.
5·91
2·33
3·19
5·73
1·99
2·64
= Monthly Rainfall
Number of deaths from E.C.F.
Percentages of deaths from E.C.F.
Average number of R. appendiculatus on ears per animal
" " R. evertsi under base of tail per animal